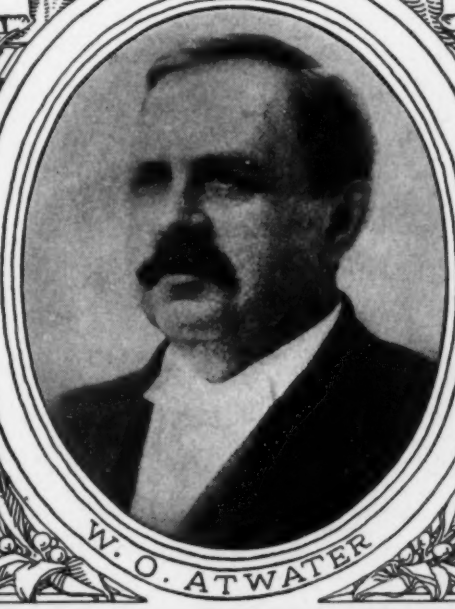
JANUARY 10, 1945

Lewis

THE JOURNAL OF NUTRITION

VOLUME 29 NUMBER 1



GEORGE R. COWGILL, *Editor*
Yale University School of Medicine, New Haven 11, Conn.

EDITORIAL BOARD

L. C. NORRIS
HOWARD B. LEWIS
ALFRED T. SHOHL
ROLAND M. BETHKE
GEORGE O. BURR
FRED A. HITCHCOCK
CONRAD A. ELVEHJEM
E. M. NELSON
RUSSELL M. WILDER
O. A. BESSEY
F. C. BING
W. J. DANN

Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA 4, PA.

Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

Entered as second-class matter January 20, 1934, at the post office of Philadelphia, Pa., under the Act of March 3, 1879. Copyright 1945. The Wistar Institute of Anatomy and Biology. All rights reserved.

Publications of The Wistar Institute

JOURNAL OF MORPHOLOGY

Devoted to the publication of original research on animal morphology and physiology, including cytology, protozoology, and the embryology of vertebrates and invertebrates. Articles do not usually exceed 50 pages in length.

Issued bimonthly, 2 vols. annually: $20.00 Domestic, $21.00 Foreign, per year.

THE JOURNAL OF COMPARATIVE NEUROLOGY

Publishes the result of original investigations on the comparative anatomy and physiology of the nervous system.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE AMERICAN JOURNAL OF ANATOMY

Devoted primarily to the interests of anatomy as taught in the medical schools of the United States and to the development of scientific anatomy in these schools. Publishes researches only.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE ANATOMICAL RECORD

Organ of the American Association of Anatomists and the American Society of Zoologists

For the prompt publication of concise original articles on vertebrate anatomy, preliminary reports; technical notes; critical notes of interest to anatomists and short reviews of noteworthy publications.

Issued monthly, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

THE JOURNAL OF EXPERIMENTAL ZOOLOGY

Publishes papers embodying the results of original researches of an experimental or analytical nature in the field of zoology.

Issued 9 times a year, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY

Organ of the American Association of Physical Anthropologists

Publishes original articles on comparative human morphology and physiology as well as on the history of this branch of science and the techniques used therein. In addition it gives comprehensive reviews of books and papers, an annual bibliography, and informal communications.

Issued quarterly, 1 vol. annually: $6.00 Domestic, $6.50 Foreign, per year.

JOURNAL OF CELLULAR AND COMPARATIVE PHYSIOLOGY

Publishes papers which embody the results of original research of a quantitative or analytical nature in general and comparative physiology, including both their physical and chemical aspects.

Issued bimonthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE JOURNAL OF NUTRITION

Organ of the American Institute of Nutrition

Publishes original researches in the field of nutrition and occasional reviews of literature on topics with which the journal is concerned.

Issued monthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE AMERICAN ANATOMICAL MEMOIRS

Publishes original monographs based on experimental or descriptive investigations in the field of anatomy which are too extensive to appear in the current periodicals. Each number contains only one monograph. List of monographs already published, with prices, sent on application.

ADVANCE ABSTRACT CARD SERVICE

Every paper accepted for publication in one of the above periodicals is accompanied by the author's abstract. The abstract and the complete bibliography reference to the paper as it will eventually appear is printed on the face of a standard library catalogue card. This Advance Abstract Card Service is issued promptly, in advance of the journal containing the paper, and is offered in three styles.

Prices per year $2.00, $2.50 and $3.00, postpaid.

These publications enjoy the largest circulation of any similar journals published.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

WOODLAND AVENUE AND THIRTY-SIXTH STREET, PHILADELPHIA 4, PA.

ACUTE AND CHRONIC BIOTIN DEFICIENCIES IN THE MONKEY[1] (MACACA MULATTA)

HARRY A. WAISMAN, KEITH B. McCALL AND C. A. ELVEHJEM
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

THREE FIGURES

(Received for publication July 24, 1944)

Biotin deficiency has been produced in animals through the addition of raw egg white or powdered raw egg white to several different diets (Boas, '27; Parsons, '31; Salmon and Goodman, '34; György, '35). Landy ('41) has reviewed the early literature dealing with the experimental and clinical use of egg white in the diet. Recent studies have demonstrated the need for biotin in the diet of certain animals without the use of a biotin-inactivating substance (Hegsted et al., '40, '42; Waisman et al., '42; Ansbacher and Landy, '41; Patrick et al., '41; Cooperman et al., '43; and Waisman and Elvehjem, '43). In the experiments described in this report, biotin deficiency has been produced in the monkey by three methods: (1) limiting the biotin intake over long periods, (2) including egg white in a complete diet, and (3) adding a sulfonamide drug to an adequate diet.

CHRONIC BIOTIN DEFICIENCY

Waisman et al. ('43) demonstrated that monkeys grow normally on a purified diet containing 3% whole liver substance (W.L.S.), liver concentrate powder 1:20 (L.E.), or a solubilized liver residue (fraction L). Fairly good growth was still obtained when the W.L.S. and L.E. were reduced to 1%, but nutritional failure resulted when only 1% fraction L was fed. These results can be correlated with the amount of "folic acid" (Mitchell et al., '41) or "norite eluate factor" (Hutchings et al., '41) present in the supplement (Waisman and Elvehjem, '43).

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the National Foundation for Infantile Paralysis, Inc., New York.

We are indebted to The Wilson Laboratories, Chicago, for the liver products; to Dr. A. D. Welch of Sharp and Dohme, Glenolden, Pa., for the sulfa drugs; to Abbott Laboratories, North Chicago, for the halibut liver oil; and to Merck and Co., Rahway, New Jersey, for the crystalline vitamins.

Copyright, 1945
The Wistar Institute of Anatomy and Biology.
All rights reserved.

In the present experiments, animals fed for 6 to 15 months on a basal diet consisting of sucrose 73 parts, purified casein 18, corn oil 3, salts 4, cod liver oil 2, together with the daily addition of thiamine 500 μg., riboflavin 1 mg., pyridoxine 1 mg., calcium pantothenate 3 mg., nicotinic acid 10 mg., choline 100 mg., para-aminobenzoic acid 100 mg., inositol 100 mg., and 25 mg. vitamin C plus 3% fraction L usually showed a thinning of the hair and loss of fur color. Loss of the hair coat may precede the gradual changes in the hair color or vice versa, and may be evenly distributed or limited to definite areas on the shoulders, back or rump. Five monkeys on the same basal diet, but with 3% L.E. showed a less rapid loss of the hair than those fed the fraction L diets. A male animal, monkey no. 1, given the 3% L.E. diet, showed excellent growth over a 33-month period (starting weight 2300 gm., present weight 13,500 gm.), but during the fifteenth and eighteenth months a gradual loss of the fur was observed; after 24 months the remaining fur was uniformly distributed but the color was abnormal; no further hair loss was noted for the next 6 months. This animal was then given 40 μg. biotin methyl ester per day, and after a month there was marked improvement in the fur color on the legs and lower back. Monkeys fed the same purified diet, but with 3% W.L.S. have shown no changes in the fur during a period of 28 months.

The biotin content of the various liver fractions was determined [2] by microbiological assay according to procedures outlined by Shull et al. ('42) and by Lampen et al. ('42). The assays showed that W.L.S. contained 2–3 μg. biotin, L.E. 0.29–1.5 μg. and fraction L 0.23–0.57 μg. per gm. Since the average daily food consumption of the animals is approximately 200 gm. of the 3% liver diets, such monkeys would receive about 6 gm. of the respective liver fractions per day, or 12–18 μg. biotin from the W.L.S. diet, 1.7–9.0 μg. from the L.E. diet, and 1.3–3.4 μg. from the fraction L diet. From the results of 3 years observations on monkeys receiving various liver diets, it can be concluded that the fur loss is directly related to the biotin potency of the various liver products. The present experiments have established that 20 μg. crystalline biotin methyl ester per day is capable of restoring normal hair growth and fur color in monkeys which have been fed for 15 to 24 months a diet containing suboptimal amounts of biotin (figs. 1 and 2). No attempt has been made to determine the exact biotin requirement; but during a portion of this study, 13.8 μg. were fed per day with partial responses in hair growth, and when the biotin was increased to 20 μg. a noticeable improvement resulted.

[2] Several biotin assays were kindly made by Dr. J. Gardner, Dr. J. O. Lampen and Mr. D. Miller.

In a previous paper (Waisman and Elvehjem, '43) the experimental findings on two animals which received 1% fraction L, but no added calcium pantothenate, were described in detail. One lost its fur within 150 days; when subsequently fed 3% fraction L, the weight increased, but the fur was still sparse. When the other monkey received "folic acid" concentrate equivalent to 5 gm. fraction L, but no biotin, the fur coat improved temporarily, but after 2 to 3 months large denuded areas reappeared, indicating that the "folic acid" concentrate was of

1

2

Fig. 1 Monkey no. 61. Fed low biotin diet (M — 3 + 1% fraction L) (chronic deficiency).
Fig. 2 Monkey no. 61. Three months after feeding 20 μg. biotin methyl ester.

little value in permanently restoring the fur. The "folic acid" concentrate or higher levels of fraction L, were able only to delay the early loss of fur.

Gradual loss of fur has also been noted in monkeys maintained on a thiamine low diet (Waisman and McCall, '44) for several months. A bisulfite-treated liver extract was included in the diet to provide "folic acid" and other unknown factors. The bisulfite treatment reduced the thiamine content of the L.E. to a barely detectable amount, and had little destructive action on the biotin content as shown by microbiological assay. The fur loss, however, was noted much sooner (8–12

months) than in animals which received the untreated liver extract. This was due in part to the decreased food consumption in those animals maintained on borderline levels of thiamine.

Less marked chronic biotin deficiency was manifested by the appearance of the typical "rusty deposit" (presumably porphyrin-like substances — (McElroy et al., '41; Chick et al., '40) on the abdomen and groin. No skin changes or loss of fur were observed during the course of a year's study in those thiamine deficient animals in which "folic acid" concentrate, and crystalline biotin were substituted for the bisulfite-treated liver.

A definite relationship exists between hair growth and hormonal balance in monkeys. Fur loss was rapid during the attainment of puberty and menstruation in the females fed a biotin-low diet for long periods. When crystalline biotin was fed, female animals which had been undergoing sexual changes and had reached puberty showed a slower regrowth of fur than did immature females or males. In several adult females, the growth of hair seemed to follow the area of extreme redness, and the fur was much more abundant about the hips and legs than on the back and arms. In females with extensive hyperemic menstrual folds along the sides of the legs and around the hips, the hair on this area was never lost completely. Monkey no. 16 had reached puberty shortly after arrival in the laboratory and throughout 2 years of subsistence on various liver extract diets showed marked hyperemia of the eyebrows and continual redness of the perianal region. After a year or more on 3% L.E. the fur became dull and grayish looking, and 6 months later the animal had a sparse fur coat. Twenty micrograms of crystalline biotin were fed daily at this time, and after 2–3 months, a definite return of hair growth and color was evident. Four months later this animal still had redness on the bony prominences around the eyebrows and the hair had regained its color, lustre, and texture. Throughout the long course of biotin therapy, the sexual changes and menstrual folds persisted in intensity as before, but the time necessary for fur regrowth in this animal was undoubtedly related to the increased hormonal activity. Similar observations have been made in six female monkeys.

ACUTE BIOTIN DEFICIENCY

Egg white experiments

The earliest observations on the effect of dried egg white in experimental diets were made about 1927 by several groups of workers and a review of the literature on this subject has been presented by Landy

('41). Three independent and parallel lines of investigation, which dealt with the growth factors required by both rats and bacteria, finally merged so that the relationship among vitamin H, coenzyme R and biotin was clarified (György et al., '40). The review by du Vigneaud ('42) furnishes a concise presentation of the early literature and historical development of the subject.

Early experiments on egg white "toxicity" were in part complicated by diets inadequate in several factors. With this in mind our monkeys on the egg white diet received adequate quantities of all the known vitamins and all the unknown factors which could be supplied by L.E. A diet consisting of sucrose 69 parts, powdered hens' egg albumin 10,

Fig. 3 Monkey no. 83. Acute biotin deficiency (M — 3 + 2% L.E. + 10% egg white).

purified casein 10, L.E. 2, salts 4, corn oil 3, cod liver oil 2, thiamine 500 μg., riboflavin 1 mg., pyridoxine 1 mg., calcium pantothenate 3 mg., nicotinic acid 10 mg., choline 100 mg., para-aminobenzoic acid 100 mg., inositol 100 mg., and ascorbic acid 25 mg. was given to six monkeys nos. 49, 83, 84, 85, 104, and 105. Three of these animals showed signs of acute biotin deficiency within 55 to 100 days; one showed dematitis at 21 days, and two others showed symptoms after the 105th day on the diet. The acute deficiency gave a more severe dermatitis around the face, hands, and feet than was observed in the chronic deficiency (fig. 3). The eyes watered excessively and became red, and incrustations

appeared on the eyelids. Some loss of fur color was observed in those monkeys fed the egg white diet for as long as 4 months. Food intake was normal at first but decreased after several weeks. A loss in weight occurred only after the first signs of dermatitis appeared. These signs of acute biotin deficiency are identical with those described by Lease et al. ('37) in two monkeys fed 20% and 40% egg white. The removal of the egg white from the diets given our monkeys and restoration of purified casein to a level of 18% caused a gradual return to normal. Improved appetite was followed by a slow clearing of the dermatitis of the face, hands, and feet, until a new smooth skin appeared in the affected area; the fur color returned to normal and the body weight increased.

The daily administration of 20 μg. of crystalline biotin methyl ester to monkeys receiving egg white caused a rapid return to normal. Eakin et al. ('40) showed that 1 gm. of egg white is capable of inactivating approximately 2.2 μg. of pure biotin; so that the 10% egg white in the diet theoretically makes 22 μg. unavailable to the animal. The addition of 20 μg. of crystalline biotin was evidently sufficient to overcome the harmful action of that quantity of egg white in the diet.

Monkeys which had acute biotin deficiency seemed susceptible to intercurrent infection since various necrotic foci around the face, and at the margin of the teeth and gums persisted throughout the period of egg white feeding. Monkey no. 49 had a small infection of the upper right gum, which gradually became necrotic as the deficiency progressed. In monkey no. 84, a deep crack appeared in the dermatitis of the left leg just below the ankle, which became infected, but improved quickly after daily administration of 20 μg. of biotin. Caldwell and György ('43) and Trager ('43) have recently studied the role of biotin in resistance to infection.

Egg white injury in man has been described by Sydenstricker and coworkers ('42) who found a grayish pallor of the skin, anemia, atrophy of the lingual papillae, and increasing dryness of the skin, which progressed to a maculosquamous dermatitis of the neck, hands, arms, and legs. Decreased urinary biotin excretion was observed after 7 weeks on the egg white diet and neurological signs were also prominent. All these symptoms regressed after administration of 75 to 300 μg. of biotin in the form of a commercial concentrate. Rhoades and Abels ('43) found no signs of biotin deficiency in two patients fed 1000–1200 avidin units (375 gm. frozen egg white and 165 gm. dried egg white) per day for 30 weeks. Oppel ('42) has shown that the biotin content of the urine is influenced by the amount of biotin in the diet, and that

the total biotin output in humans is 3–6 times as great as the intake from the diet.

Sulfa drug experiment

Sulfa drugs have been used in nutritional experiments to inhibit the growth of intestinal bacteria, and thereby prevent bacterial synthesis of certain dietary essentials which are in turn utilized by the host (Black et al., '42; Daft et al., '42; Ransone and Elvehjem, '43). Both sulfaguanidine and sulfasuxidine (succinylsulfathiazole) have been used in attempts to negate the influence of intestinal synthesis, and thus produce one or more dietary deficiencies in a shorter time.

Monkey no. 28 received 1% sulfaguanidine or 1% sulfasuxidine at different times, and each drug retarded growth with equal effectiveness. The loss in body weight was overcome by feeding a "folic acid" concentrate; but after a period of 11 months signs of chronic biotin deficiency were observed, since no biotin was added to the diet at any time; this hair loss was accompanied by the "rusty" secretion on the abdomen and a distinct dryness of the skin. Monkey no. 32 received 1% sulfaguanidine plus 3% L.E. in addition to the basal diet for nearly 7 months, at which time the liver extract was discontinued. The 1% sulfaguanidine was fed for another 2 months with no change in the condition of the animals. One per cent sulfasuxidine was now substituted for the sulfaguanidine and after 3 months the fur color altered and the hair began to fall out. The L.E. feeding presumably deferred the loss of hair. Areas of denudation were accompanied by dryness of the skin and slight reddening of the eyes similar to mild signs of deficiency. It appears that either 1% sulfasuxidine or 1% sulfaguanidine is inadequate to give the severe signs of acute deficiency observed when egg white is fed.

Monkeys nos. 49 and 83 received the basal diet with 1% liver extract and 1% sulfasuxidine plus a daily vitamin supplement without para-aminobenzoic acid (PABA). Monkey no. 83 gained weight consistently, and attained puberty normally during this period, although a definite dryness of the hands and feet was seen at the end of 5 months. The control animal, no. 49, which received 20 μg. of biotin, was normal in appearance and showed improvement of the fur.

Monkeys nos. 84 and 85 received the basal diet plus 1% liver extract and 3% sulfasuxidine without PABA; within 2½ months dryness of the skin appeared on the mouth, nose, arms, and legs, followed 2 weeks later by severe incrustations of the skin. The animals gradually lost appetite, and after 4 months on the diet, deep cracks appeared in the

heavy scaly dermatitis of the face. Administration of 20 μg. of crystalline biotin methyl ester per day to monkey no. 85 gave a dramatic cure of all signs of the dermatitis within a 2–3-week period. New-looking smoooth skin appeared as the incrustations fell off, leaving an area through which the skin markings were clearly visible. After 2 months of biotin therapy this monkey grew a new coat of fur. Monkey no. 84 received 100 mg. of PABA per day; but 30 days later showed only slight improvement and after 60 days, the dermatitis was worse and blood appeared at the cracks on the face.

Black et al. ('42) found that PABA counteracted the effect of ½% sulfaguanidine fed in the diet of rats, and Welch et al. ('43) showed that PABA is unable to overcome the effect of sulfasuxidine in rats. PABA is also unable to counteract the effect of sulfasuxidine in the diet of monkeys as seen from the data given in the present report. Although the diet contained only 1% liver extract as a source of "folic acid" it seems unlikely from other data that insufficient "folic acid" would affect the course of the biotin deficiency disease. Experiments are now in progress in which 3% sulfasuxidine is fed together with "folic acid," equivalent to 10% solubilized liver extract.

Complete blood counts [3] were made on the monkeys receiving sulfa drugs in the diet, but no significant changes were noted in the hemoglobin, red blood cell, total white blood cell, and differential white cell determinations during acute biotin deficiency. Axelrod et al. ('43) and Spicer et al. ('42) found granulocytopenia in rats receiving sulfa drugs but their animals were on a synthetic diet without any liver fractions.

The prothrombin time [4] of 12.5% plasma from monkeys fed 1% and 3% sulfasuxidine was not appreciably changed from the normal values. This finding is in contrast to the increased prothrombin time found in rats fed 0.5% sulfaguanidine or sulfasuxidine by Black et al. ('42) and Welch et al. ('43); however, our monkeys were receiving liver fractions.

The addition of 1% sulfasuxidine to the diet, did not seem to aid the monkeys in resisting intercurrent infections which usually accompany the "folic acid" deficiency (Waisman and Elvehjem, '43). Two of the animals fed the drug with the basal diet showed signs of the deficiency, and as the disease progressed the gums became infected and small foci of necrosis were apparent at the tooth line; with the administration of "folic acid" the infection improved.

[3] The authors are indebted to Mrs. Edith S. Jones for help in the blood studies and to Mr. Jack Cooperman for his cooperation in parts of these studies.

[4] We are indebted to Dr. John B. Field and Professor K. P. Link for determining the prothrombin time in our monkeys.

DISCUSSION

The role of intestinal bacterial synthesis in providing biotin to the host is a factor that must be recognized, but since biotin deficiency does occur after long periods it is evident that inadequate amounts of biotin are made available through this means. Intestinal synthesis may also be a factor in the acute biotin deficiency which is produced by feeding 10% egg white. The severe dermatitis is gradually improved after the egg white is removed from the diet, indicating that biotin is slowly synthesized in the tract. The intestinal bacteria were presumably active and unchanged, but the synthesized biotin was immediately made unavailable by the presence of avidin in the egg white. The amount of biotin necessary for normal body function and for the prevention of fur loss is approximately 20 μg. per day. Intestinal bacteria are evidently unable to supply the total daily biotin requirement.

The ability of "folic acid" concentrates to delay or temporarily nullify the antagonistic action of the sulfa drugs has not been adequately explained. "Folic acid" preparations contain PABA which overcomes sulfaguanidine effects, but "folic acid" itself, may be a specific antagonist to sulfasuxidine action. On the other hand "folic acid" may be simply the essential factor for bacterial growth in the presence of sulfasuxidine so that small amounts of biotin may be synthesized and available to the host. Biotin can overcome the host growth inhibition of sulfa drugs in rats (Nielsen and Elvehjem, '42), but it is not known whether biotin is the missing factor per se, or whether biotin is a growth essential for intestinal bacteria enabling synthesis of still another substance required for growth of the host, or fur growth in the case of monkeys. Another point of interest in the problem of vitamin interrelationships is the observation that chronic biotin deficiency appears to be unaffected by a simultaneous pantothenic acid deficiency; the loss of fur is neither hastened nor retarded in the combined deficiency.

Our observations on the relationship of fur growth and hormonal balance in monkeys find support in the observations of Gardner and DeVita ('40), Mulligan ('43), and in Queries and Minor Notes (J. Am. Med. Assn., vol. 121, p. 274, 1943).

The loss of fur in chronic biotin deficient monkeys occurs throughout the year, and the season appears to be without influence on the denudation or regrowth of the fur.

SUMMARY

A chronic biotin deficiency in the monkey produces a thinning of the fur coat and a gradual loss of color in the hair. The time elapsing

before the hair loss in monkeys fed whole liver, liver extract, and solubilized livers can be correlated with the biotin content of the respective liver diets. Twenty μg. crystalline biotin per day was sufficient to cure or to prevent the denudation.

The hair loss is independent of the season, but is affected by hormonal influences, as indicated by slower regrowth of fur in menstruating and adolescent females.

The acute biotin deficiency produced by feeding egg white is strikingly similar to the acute deficiency which results when 3% succinylsulfathiazole is included in a complete diet. Heavy, scaly, dermatitis covers the whole body in the later stages, but is most conspicuous on the face, arms and legs. Twenty micrograms of biotin per day was sufficient to overcome the deleterious effects of the egg white and of the sulfa drugs.

LITERATURE CITED

Ansbacher, S., and M. Landy 1941 Biotin and scaly dermatosis of the chick. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 3.

Axelrod, A. E., Paul Gross, M. D. Bosse and K. F. Swingle 1943 Treatment of leucopenia and granulopenia in rats receiving sulfaguanidine in purified diets. J. Biol. Chem., vol. 148, p. 721.

Black, S., R. S. Overman, C. A. Elvehjem and K. P. Link 1942 Effect of sulfaguanidine on rat growth and plasma prothrombin. J. Biol. Chem., vol 145, p. 137.

Boas, M. A. 1927 Effect of dessication upon the nutritive properties of egg white. Biochem. J., vol. 21, p. 712.

Caldwell, F. E., and P. György 1943 Effect of biotin deficiency on duration of infection with Trypanosoma lewisi in the rat. Proc. Soc. Exp. Biol and Med., vol. 53, p. 116.

Chick, H. T., H. Macrae and A. N. Worden 1940 Relation of skin lesions in the rat to deficiency in the diet of different B_2 vitamins. Biochem. J., vol. 34, p. 580.

Cooperman, J. M., H. A. Waisman and C. A. Elvehjem 1943 Nutrition of the Golden Hamster. Proc. Soc. Exp. Biol. and Med., vol. 52, p. 250.

Daft, F. S., L. L. Ashburn and W. H. Sebrell 1942 Biotin deficiency and other changes in rats given sulfanylguanidine or succinylsulfathiazole in purified diets. Science, vol. 96, p. 321.

Eakin, R. E., E. E. Snell and R. J. Williams 1940 A constituent of raw egg white capable of inactivating biotin in vitro. J. Biol. Chem., vol. 136, p. 3.

Gardner, W. V., and J. DeVita 1940 Inhibition of hair growth in dogs receiving estrogens. Yale J. Biol. and Med., vol. 13, p. 213.

György, P. 1935 Handbuch der kinderheilkunde, Pfaundler and Schlosman, Berlin, 4th edition, vol. 10, p. 45.

György, P., D. B. Melville, D. Burk and V. du Vigneaud 1940 Possible identity of vitamin H with biotin and coenzyme R. Science, vol. 91, p. 243.

Hegsted, D. M., R. C. Mills, G. M. Briggs, C. A. Elvehjem and E. B. Hart 1942 Biotin in chick nutrition. J. Nutrition, vol. 23, p. 175.

Hegsted, D. M., J. J. Oleson, R. C. Mills, C. A. Elvehjem and E. B. Hart 1940 Studies on a dermatitis in chicks distinct from pantothenic acid deficiency. J. Nutrition, vol. 20, p. 599.

HUTCHINGS, B. L., N. BOHONOS AND W. H. PETERSON 1941 Growth factors for bacteria XIII. Purification and properties of an eluate factor required by certain lactic acid bacteria. J. Biol. Chem., vol. 141, p. 521.

LAMPEN, J. O., G. P. BAHLER AND W. H. PETERSON 1942 The occurrence of free and bound biotin. J. Nutrition, vol. 23, p. 11.

LANDY, M. 1941 "Vitamin H., biotin and coenzyme R, a brief review of the literature." Research Laboratories, SMA Corp., Chagrin Falls, Ohio.

LEASE, J. G., H. T. PARSONS AND E. KELLY 1937 A comparison in five types of animals of the effects of dietary egg white and of a specific factor given orally or parenterally. Biochem. J., vol. 31, p. 433.

MCELROY, L. W., K. SOLOMON, F. H. J. FIGGE AND G. R. COWGILL 1941 On the porphyrin nature of the fluorescent "blood caked" whiskers of pantothenic acid deficient rats. Science, vol. 94, p. 467.

MITCHELL, H. K., E. E. SNELL AND R. J. WILLIAMS 1941 Concentration of "folic acid." J. Am. Chem. Soc., vol. 63, p. 2284.

MULLIGAN, R. M. 1943 Hair loss in male dogs fed stilbestrol. Proc. Soc. Exp. Biol. and Med., vol. 54, p. 21.

NIELSEN, E., AND C. A. ELVEHJEM 1942 Cure of paralysis in rats with biotin concentrates and crystalline biotin. J. Biol. Chem., vol. 144, p. 405.

OPPEL, F. W. 1942 Studies of biotin metabolism in man. Am. J. Med. Sciences, vol. 204, p. 856.

PARSONS, H. T. 1931 Physiological effects of diets rich in egg white. J. Biol. Chem.. vol. 90, p. 351.

PATRICK, H., R. V. BOUCHER, R. ADAMS DUTCHER AND H. C. KNANDEL 1941 Biotin and prevention of dermatitis in turkey poults. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 456.

RANSONE, B., AND C. A. ELVEHJEM 1943 Value of biotin, folic acid concentrates and liver extract in the diet of rats fed succinylsulfathiazole. J. Biol. Chem., vol. 151, p. 109.

RHOADES, C. P., AND J. C. ABELS 1943 The administration of egg white and avidin concentrates to patients with cancer. J Am. Med. Assn., vol. 121, p. 1261.

SALMON, W. D., AND J. G. GOODMAN 1934 Studies of the raw egg white syndrome in rats. J. Nutrition, vol. 8, p. 1.

SHULL, G. M., B. L. HUTCHINGS AND W. H. PETERSON 1942 A microbiological assay for biotin. J. Biol. Chem., vol. 142, p. 913

SPICER, S. S., F. S. DAFT, W. H. SEBRELL AND L. L. ASHBURN 1942 Prevention and treatment of agranulocytosis and leucopenia in rats given sulfanylguanidine or succinylsulfathiazole in purified diets. U. S. Public Health Reports, vol. 57, p. 1559.

SYDENSTRICKER, V. P., S. A. SINGAL, A. P. BRIGGS, N. M. DEVAUGHN AND H. ISBELL 1942 Observations on the "egg white injury" in man, and its cure with a biotin concentrate. J. Am. Med. Assn., vol. 118, p. 1199.

TRAGER, W. 1943 Influence of biotin upon susceptibility to malaria. J. Exp. Med., vol. 77, p. 557.

DU VIGNEAUD, V. 1942 The biological action of the vitamins. University of Chicago Press, Chicago, Ill., p. 144.

WAISMAN, H. A., AND C. A. ELVEHJEM 1943 The role of biotin and "folic acid" in the nutrition of the rhesus monkey. J. Nutrition, vol. 26, p. 361.

WAISMAN, H. A., AND K. B. MCCALL 1944 A study of thiamine deficiency in the monkey (Macaca mulatta). Arch. of Biochem., vol. 4, p. 265.

WAISMAN, H. A., R. C. MILLS AND C. A. ELVEHJEM 1942 Factors required by chicks maintained on a heated diet. J. Nutrition, vol. 24, p. 187.

WAISMAN, H. A., A. F. RASMUSSEN, JR., C. A. ELVEHJEM AND P. F. CLARK 1943 Studies on the nutritional requirements of the rhesus monkey. J. Nutrition, vol. 26, p. 205.

WELCH, A. D., L. D. WRIGHT AND K. L. SPRAGUE 1943 Role of "folic acid" and biotin in the nutrition of the rat. J. Nutrition, vol. 25, p. 555.

THE IMPORTANCE OF COMMERCIAL PROCESSING FOR THE PROTEIN VALUE OF FOOD PRODUCTS

I. SOYBEAN, COCONUT AND SUNFLOWER SEED

H. H. MITCHELL, T. S. HAMILTON AND J. R. BEADLES,
WITH THE TECHNICAL ASSISTANCE OF FRANK SIMPSON
Division of Animal Nutrition, University of Illinois, Urbana

(Received for publication August 23, 1944)

In studies of the protein values of food products the emphasis seems to be shifting from the protein values of individual foods to the effect upon these values of the commercial processes used in preparing them for human consumption and in storing them for future use. It is true that individual foods show wide differences in the nutritive value of their proteins. Expressed in terms of biological values as determined upon growing rats at a dietary level of approximately 10%, the proteins of the many foods studied range from 40 to 95. But when combined into diets as complicated as are commonly consumed in this country, the biological values of the proteins of such mixed diets, when reasonably well balanced, may be expected to be much less variable, perhaps falling generally, in the same terms, between 60 and 75. In other words, the combination of foods into diets has a leveling effect on the protein quality, but in such a way that the quality of the mixed proteins will be higher than the mean quality of the individual proteins to the extent that supplementary effects operate.

The most important agency modifying the nutritive value of the proteins of foods during processing is heat. The effect of processes involving heat upon the digestibility and biological value of protein is especially important in human nutrition, but whether the effect of heat will be large or small, or whether it will impair or increase the nutritive value of a given protein source is quite unpredictable on the basis of available information.

The sensitivity of cereal proteins to heat has been known since the experiments of Morgan ('31), but the subject has come into prominence recently in connection with the effect of the extreme heat, applied for only a short time, in the explosion process of preparing certain cereal breakfast foods. Stewart, Hensley and Peters ('43) have shown in feeding experiments on growing rats that the ratio of gain in weight to

protein consumed, when the food intakes of the rats are equalized, or nearly so, is depressed by two-thirds by the gun-explosion of an oats-corn-rye mixture, and by three-fourths by the gun-explosion of oats alone. Evidently the preparation of these breakfast cereals yields a product whose protein value in nutrition bears little if any relation to that of the raw cereals from which they were derived. In the public interest, it would seem to be desirable to process cereals for human consumption by methods that are less destructive of nutrient content.

The effect of heat upon the proteins of the leguminous seeds is to improve the nutritive value of the proteins, either by marked improvements in digestibility, as in the navy bean (Waterman and Johns, '21) and the velvet bean (Waterman and Jones, '21), or by a marked improvement in biological value associated with only a slight improvement in digestibility, as in the case of the soybean (Hayward, Steenbock and Bohstedt, '36, and many others), or by means hitherto uninvestigated, as in the case of the lima bean (Finks and Johns, '21) and the lentil (Jones and Murphy, '24).

In cases where heat improves the protein value in nutrition of a given food protein, evidently the intensity of the heat treatment and the time of application will determine the degree of improvement. A too severe heat treatment will undo the favorable effects of a milder treatment. The work of Mitchell, Burroughs and Beadles ('36) demonstrating the refractoriness to commercial roasting of the proteins of the peanut and the relatively low biological values of the protein of both raw and roasted peanuts, 58 and 56, respectively, is not inconsistent with the experiments of Jones and Divine ('44) indicating that expeller process peanut meal contains protein with a growth-promoting value comparable to that of expeller process soybean meal. The roasting of peanuts may represent a much more severe heat treatment than oil extraction by the expeller process, so severe as to nullify the improvement in biological value induced by a milder heat treatement.

It seems of interest to determine quantitatively the effect of heat treatment on the proteins of the soybean, and particularly the effect of the explosion process that is so destructive of the cereal proteins.

The effect of heat processing on soybean protein

The digestibility and the biological value of the protein in raw soybeans of the Illini variety and of the same sample of soybeans heated in the autoclave for 1 hour at 15 pounds steam pressure were determined by the nitrogen metabolism method used in this laboratory (Mitchell and Carman, '26), with some later modifications. These modifications

include the use of a feces marker (Fe_2O_3 or Cr_2O_3), a reversal system of feeding whereby each rat receives each of the two protein sources to be compared, five rats in one order and the other five in the reverse order with the standardizing period in between, and a system of paired feeding, treating the ten rats as five pairs within which the food intake is equalized.

The experimental rations in this test contained 12.5 to 13.1% of total protein ($N \times 6.25$), or 10 to 11% of digestible protein. In other respects they were adequate for maximum growth. The water-soluble vitamins were added as Harris' yeast concentrate.

The true digestibility and the biological values, computed in the usual way, are summarized in table 1.

TABLE 1

A comparison of the utilization of protein in raw and autoclaved soybeans.

RAT NUMBER	RAW SOYBEAN MEAL		AUTOCLAVED SOYBEAN MEAL	
	True digestibility of protein	Biological value	True digestibility of protein	Biological value
	%	%	%	%
75 m	78	52	83	72
77 m	72	48	83	63
79 m	78	47	86	70
81 m	76	45	83	62
83 f	76	49	79	76
76 m	81	52	85	67
78 m	74	44	80	66
80 m	81	52	88	64
82 m	83	51	82	64
84 f	81	51	82	66
Average	78.0	49.1	83.1	67.0

There is evidently a statistically significant difference between autoclaved and raw soybean meal in both the digestibility of the nitrogen and in its biological value. Autoclaving has increased the digestibility of the soybean nitrogen by some 5 percentage units and the biological value by 18 percentage units. It may be noted that the average results in this experiment on autoclaved soybeans check remarkably well with the results of an experiment carried out 6 years later (Mitchell and Beadles, '44) on Illini soybeans autoclaved for 1.5 hours at 17 pounds steam pressure. In the latter test the average true digestibility of nitrogen, corrected, as are the values in table 1, for the metabolic nitrogen in the feces, is 84.4%, and the average biological value, 67.5.

In paired-feeding experiments on young growing rats, it was shown by Mitchell and Smuts ('32), and later by others, that cystine is the amino acid limiting the utilization of raw soybean proteins in metabolism. In later paired-feeding experiments, the details of which need not be given here, it was shown that cystine also limits the utilization in metabolism of the autoclaved soybeans used in the tests summarized in table 1. With eight pairs of rats (45 to 50 gm. initial weight) on diets containing 10% of protein, the rat in each pair receiving a cystine supplement gained at the more rapid rate; the mean difference in gain in a 2-week feeding period was 6.4 gm., the standard deviation of differences was 2.55 gm., and the probability of a fortuitous outcome was so small (.0002) that it can safely be neglected (Student, '08).

In another series of tests with eight pairs of rats, 10% of raw soybean protein plus 0.25% of cystine was compared with 10% of autoclaved soybean protein plus 0.25% of cystine. In all pairs but one, the rat receiving autoclaved soybeans gained the faster in a 5-week feeding test. The mean difference in gain induced by the two diets was 8.1 gm., the standard deviation of differences was 5.35 gm., and the probability that a random combination of uncontrolled experimental factors could have brought about such a result is only 0.0025, and can safely be neglected. Repetition of the tests with older rats and with a supplement of 0.4% of cystine instead of 0.25% failed to reveal a significant difference in growth-promoting power of autoclaved over raw soybeans in a 2-week feeding test. The latter test was not entirely satisfactory because of the heavier initial weights of the rats, 74 to 109 gm., and the less perfect initial pairing.[1]

The conclusion seems justified, however, that the effect of the heating treatment in these particular tests was to render soybean protein more digestible and more completely utilizable in metabolism, the latter effect being due solely to an improved availability of the cystine contained in the soybean protein.

To determine the effect of the explosion process upon the nutritive value of the protein of the soybean, we obtained three samples of soybean flour all taken from the same basic lot of soybeans.[2] These samples were a raw soybean flour, an exploded soybean flour and a partially exploded soybean flour.

The raw soybean flour was not submitted to any heat treatment other than that involved in grinding.

[1] The paired-feeding tests referred to in this and in the preceding paragraph were carried out by Dr. A. J. Johanson, then a graduate student in animal nutrition.

[2] These samples were obtained through the Musher Corporation of New York City, which contributed funds for the carrying out of these tests.

In the preparation of the exploded soybean flour, the beans were placed in a rotating closed chamber, or "gun", at a temperature of approximately 550°F. for a period of 90 seconds. Steam was then injected into the gun for an additional period of 60 seconds until the steam pressure reached 185 pounds per square inch. At the end of this time, the gun was suddenly opened so as to produce an instantaneous drop in temperature and pressure to atmospheric conditions.

The partially exploded soybean flour was prepared exactly as described for the fully exploded flour except that the steam was injected over a period of 45, instead of 60 seconds, and the pressure reached was 165 pounds per square inch, instead of 185 pounds.

The chemical analysis of the three flours, summarized in table 2, revealed a remarkable similarity in composition.

TABLE 2

Chemical composition of raw and exploded soybean flours.

SAMPLE	DRY MATTER	ETHER EXTRACT	CRUDE PROTEIN	TOTAL NITROGEN	CRUDE FIBER	GROSS ENERGY
	%	%	%	%	%	*cal./gm.*[1]
Soybean flour — Exploded	94.81	21.90	39.62	6.34	2.51	5408
Soybean flour — Raw	93.20	20.70	39.62	6.34	2.49	5339
Soybean flour — Partly exploded	94.56	22.96	39.69	6.35	1.65	5536

[1] Small calorie per gram.

The true digestibility and the biological value of the nitrogen in the three flours was compared in each case with those of a defatted and dehydrated beef, prepared at low temperatures. Ten young growing rats, weighing initially 50 to 55 gm. each, were used in each comparison, which consisted of three collection periods. In the first period, five rats received the soy flour ration and their pair mates received the beef ration. In the second period, all rats received the 4% egg protein diet as a standardizing ration, and in the third period the beef and soy flour rations were again compared but in reverse order, so that in the first and third periods each rat was tested with both rations. Throughout these periods pair mates consumed the same amount of food. Each collection period was preceded by a period of at least 5 days on constant food.

The composition of the experimental diets is given in table 3. They were equalized in fiber and energy content, and except for the standardizing egg-protein diet, in nitrogen content. The true digestibility and the biological values of the nitrogen of the three flours are collected in table 4.

The almost identical average values for the digestibility and biological value of the protein of beef in the three experiments testifies to the accuracy of the method, discussed in a previous publication (Mitchell, Burroughs and Beadles, '36), and the essential accuracy of the conception of protein metabolism upon which the method is based (Mitchell, '42; '44). The shortness of the metabolism period involved in this method has been recently criticized, because of the possibility "that a suboptimal concentration of a particular amino acid in the protein, which

TABLE 3

The experimental diets in the soybean flour tests.

CONSTITUENTS	BEEF RATION	EGG STANDARDIZING RATION	SOY FLOUR, EXPLODED, RATION	SOY FLOUR, PARTLY EXPLODED, RATION	SOY FLOUR, RAW, RATION
Dried defatted beef round	11.83	..	..	..	..
Dried defatted whole egg	..	5.75	..	..	..
Soy flour, exploded	..	..	25.24	..	..
Soy flour, partly exploded	..	..	..	25.20	..
Soy flour, raw	..	..	..	..	25.24
Salt mixture no. 446	4	4	4	4	4
NaCl	1	1	1	1	1
$BaSO_4$ (or wood flock)	2	2	1.37	1.58	1.37
Vitaminized starch [1]	5	5	5	5	5
Starch	54.83	60.58	46.92	47.01	46.61
Fortified cod liver oil	1.5	1.5	1.5	1.5	1.5
Wheat germ oil	0.5	0.5	0.5	0.5	0.5
Soy oil	5	5	..	..	..
Lard	4.34	4.67	4.47	4.21	4.78
Sucrose	10	10	10	10	10
Total	100.00	100.00	100.00	100.00	100.00
Crude protein content, %	9.82	4.31	10.06	10.25	10.31

[1] The vitaminized starch contained per gm., 20 μg. of calcium pantothenate, 60 μg. of pyridoxine HCl, 80 μg. of riboflavin, 50 μg. of thiamine HCl and 30 mg. of choline HCl.

would ultimately prove to be a handicap to the animal, might be without consequence for the nitrogen balance during a short test period."[3] This hypothetical situation seems inherently improbable. The amount of amino acids stored in the tissues is extremely small and its replenishment from the tissue proteins for the purpose of supplementing inadequate dietary protein would involve losses of nitrogen that would immediately be reflected in the nitrogen balance of the animal. As a matter of fact, the rat (Burroughs, Burroughs and Mitchell, '40) and other

[3] Nutrition Reviews, vol. 2, no. 7, p. 213.

TABLE 4

True digestibilities and biological values of the protein of the three soy flours, each compared with the protein of beef round.

EXPERIMENT NUMBER 335

RAT NO.	Beef round		Soy flour, exploded	
	True digestibility	Biological value	True digestibility	Biological value
11	100	76	93	65
13	100	75	92	72
15	100	69	92	66
17	100	77	93	74
19	100	76	91	77
12	99	81	95	74
14	98	82	93	71
16	98	80	96	68
18	99	85	96	73
20	98	81	93	72
Averages	99.2	78.2	93.4	71.2

EXPERIMENT NUMBER 336

RAT NO.	Beef round		Soy flour, partly exploded	
	True digestibility	Biological value	True digestibility	Biological value
21	100	74	93	77
23	100	67	94	75
25	100	70	95	80
27	100	76	96	75
29	100	84	94	78
22	100	82	95	66
24	100	81	98	79
26	99	82	96	72
28	100	80	97	72
30	100	85	98	78
Averages	100	78.1	95.6	75.2

EXPERIMENT NUMBER 339

RAT NO.	Beef round		Soy flour, raw	
	True digestibility	Biological value	True digestibility	Biological value
41	100	86	84	58
43	100	82	86	71
45	100	77	87	65
47	100	77	86	60
49	100	77	85	60
42	98	77	84	55
44	100	75	87	57
46	99	78	85	56
48	98	72	83	56
50	99	80	81	56
Averages	99.4	78.1	84.8	59.4

animals respond promptly in their balance of nitrogen to the withdrawal from the diet of an essential amino acid.

The average digestibility and biological value of the nitrogen of the raw soy flour, 84.8 and 59.4, respectively, were significantly lower than those of the fully exploded flour, 93.4 and 71.2, respectively. This relationship proves that the more severe explosion treatment has definitely improved the nutritive value of the soybean protein to even a greater extent than the autoclaving practiced in the preceding soybean experiments.

The protein of the partially exploded soy flour is definitely more digestible than that of the fully exploded flour, 95.6 as compared with 93.4, with a probability of only 0.021 (Fisher, '28) that chance factors alone could have brought about this spread. The biological value of the protein in the partly exploded flour averaged higher than that of the fully exploded flour, 75.2 as compared with 71.2, but here the difference is not sufficiently distinct in comparison with the intra-group variation ($P = 0.055$) to constitute a demonstration. However, the results are highly suggestive that here also the less severe heat treatment has been definitely advantageous in producing a more favorable effect on the nutritive value of soybean protein.

Mild processing methods preserve the protein value of the coconut and sunflower seed

The commercial extraction of oil from oil-bearing seeds is ordinarily carried out by procedures that give the highest yields of oil with minimal refining losses. Little attention is paid to the nutritive value of the oil meal, which is commonly used for stock feed. Whether the oil is extracted by pressure or by solvent, high temperatures are commonly employed, either in the pretreatment of the seeds, the extraction process itself, or in the removal of the solvent. Inevitably the protein value of the residual oil meal is impaired (except for the soybean) unless special precautions are taken. Olcott and Fontaine ('44) have demonstrated the injurious effects of heat on the proteins of the cottonseed, such as probably result from commercial oil extraction. The variable results that have been reported for the biological value of the proteins of cottonseed meal, ranging from 62 (Nevens, '21), through 78 (Braman, '31–'32) to 81 (Smuts and Malan, '38), may be explainable by variations in the heat treatment employed in commercial oil extraction.

The same situation appears to exist for the coconut and the effect of oil extraction on the protein value of coconut meal. In 1919, Johns, Finks and Paul reported on the growth-promoting value for rats of the

proteins of coconut press cake, and found that diets containing 13.1% of coconut protein (N × 5.7) were capable in some cases of supporting a growth rate of 1.5 to 2.0 gm. daily, which at that time was considered normal. Somewhat later, Maynard and Fronda ('21) gave to the proteins of coconut oil meal a value somewhat higher than that shown by the proteins of corn, measuring these values as gains per gram of protein consumed in ad libitum feeding experiments. However, the values reported on both foods were few and variable. Mitchell and Villegas ('23), using the nitrogen balance method, found an average biological value of 58 for the proteins of coconut meal, quite comparable with the values commonly found for corn by this method (Mitchell, '24). Using the same method, Smuts and Malan ('38) reported an average biological value of 69 for the proteins of "coprameal".

The opportunity of testing the protein value of a coconut meal that was defatted by a solvent extraction method involving low temperatures throughout (less than 75°C.) was afforded us through the courtesy of Mr. Ezra Levin of Monticello, Illinois.[4] The meal was finely ground, very light in color and possessed only a slight coconut odor. At the same time we secured also a sample of sunflower seed meal prepared by the same process and by the same company. Both meals appeared to be edible, capable of being incorporated in flour mixtures for baking. Their chemical composition is given in table 5.

TABLE 5

Chemical composition of the sunflower seed meal and the coconut meal.

	DRY MATTER	ETHER EXTRACT	CRUDE PROTEIN	ASH	CRUDE FIBER	N-FREE EXTRACT	GROSS ENERGY
	%	%	%	%	%	%	*cal./gm.*
Sunflower seed meal	95.41	4.48	52.83	6.46	4.05	27.59	4.57
Coconut meal	95.21	10.25	19.69	5.19	9.39	50.69	4.31

Both meals were incorporated into rations containing approximately 10% of protein (N × 6.25) and compared by the usual procedure, as explained above, with the proteins of beef, using young albino rats as subjects. The true digestibilities and the biological values secured are summarized in table 6.

Noteworthy, is the high average biological value of 71 for the coconut meal protein, as compared with the value of 58 previously obtained from this laboratory on a commercial meal presumably subjected to a much higher heat treatment. The average biological value of 64.5 for the pro-

[4] Of the VioBin Corporation, which provided funds to cover in part the expenses of this study.

teins of sunflower seed meal places this protein source in the same class as the better cereals, oats, wheat and barley. The digestibility of sunflower seed proteins is definitely higher, 94.3, than that of coconut proteins, 86.1, but even the latter is not unsually low, as compared with cereal proteins, for example.

TABLE 6

The digestibility and biological value of the protein of sunflwer seed meal and coconut meal, compared with the protein of beef round.

EXPERIMENT NUMBER 342

RAT NO.	Beef round		Sunflower seed meal	
	True digestibility	Biological value	True digestibility	Biological value
51	99	83	94	66
53	100	84	95	63
55	97	66	93	62
57	98	79	96	64
59	100	81	94	65
52	97	77	91	61
54	98	77	94	64
56	97	73	92	63
58	100	79	99	69
60	99	81	95	68
Averages	98.5	78.0	94.3	64.5

EXPERIMENT NUMBER 343

RAT NO.	Beef round		Coconut meal	
	True digestibility	Biological value	True digestibility	Biological value
61	99	72	87	68
63	99	74	89	70
65	99	71	88	70
67	99	69	87	66
69	94	69	88	75
62	98	60	80	70
64	97	86	86	77
66	96	77	87	71
68	98	77	87	65
70	98	83	82	75
Averages	97.7	75.8	86.1	70.7

The method of oil extraction at comparatively low temperatures, such as was used with these products, and with corn germ (Mitchell and Beadles, '44) and also wheat germ, has so many apparent advantages in preserving the nutrient content of both oil and meal that it, or some similar method, may well initiate a revolution in the extraction of

edible oils from oil bearing seeds. This will be especially true when the oil extraction business, and the consuming public, realize the high nutritive value of many oil-bearing seeds, entirely aside from their oil content. The drastic methods of oil extraction now in general use produce meals often inferior and always variable in protein value. The mild, gentle method, such as was used in the preparation of the meals here studied, produces uniform meals, stable in character, probably with the original nutrients virtually unimpaired in amount and quality,[5] and to all appearances of excellent culinary properties. They are worthy of further study to determine the extent to which they can be introduced into the human diet as sources of protein, thiamine and other nutrients.

The net protein values of the foods studied

The value of a food as a source of dietary protein is not, of course, measured by the biological value. It evidently depends upon three factors, the protein content, the digestibility of the protein and the biological value of the digested protein. Taking all of these factors into consideration, leads to what the senior author has called the "net protein" value (Mitchell and Carman, '24) which possesses obvious advantages in the comparative ranking of protein foods. The net protein values of the food products discussed in this report have been computed and summarized in table 7.

TABLE 7

The net protein values of the foods studied in these experiments, on the moisture-free basis.

FOOD PRODUCT	PROTEIN CONTENT	DIGESTIBILITY OF PROTEIN	CONTENT OF DIGESTIBLE PROTEIN	BIOLOGICAL VALUE OF PROTEIN	NET PROTEIN VALUE
	%	%	%	%	%
Raw soy flour	42.5	84.8	36.0	59.4	21.4
Partly exploded soy flour	42.0	95.6	40.2	75.2	30.2
Fully exploded soy flour	41.8	93.4	39.0	71.2	27.8
Coconut meal	20.7	86.1	17.8	70.7	12.6
Sunflower seed meal	55.4	94.3	52.2	64.5	33.7

In terms of net protein content, the products arrange themselves in the following order: sunflower seed meal, partly exploded soy flour, fully exploded soy flour, raw soy flour and coconut meal. In terms of vitamin content, the products subjected to the least severe heat treatment would probably outrank products severely heated in commercial processing.

[5] For example, the thiamine content of a corn germ meal prepared by the Levin process was found to be 25.6 μg. per gram.

SUMMARY AND CONCLUSIONS

Using the nitrogen balance method of assessing the protein values of foods with growing rats, it has been shown that the digestibility and biological value of the proteins of the soybean can be increased by heat processing, and that the explosion process, if not carried to extremes, can raise the digestibility of the protein by 11 percentage units and the biological value by 16 percentage units.

In the autoclaving of soybeans, the improvement in the nutritive value of the protein seems to be entirely referable to an improvement in the availability of the contained cystine.

In the commercial oil extraction of other oil-bearing seeds than the legumes, the drastic heat treatments commonly employed may be expected to exert a destructive action upon the heat labile nutrients, including protein. Applied to the coconut, a solvent extraction procedure carried out at temperatures that never exceed 75°C., yields a product whose protein is 86% digestible and possesses a biological value of 71, considerably higher than that of a product tested earlier that had been prepared by the usual drastic methods.

The protein of sunflower seed meal, prepared by the same process, was found to be 94.3% digestible and to possess a biological value of 64.5. Due to its high initial content of protein, 55.4% on the dry basis, the "net protein" content of this food was higher than that of any of the other foods tested, i.e., 33.7%.

The significance of a mild, gentle process of extracting oil from nonleguminous oil-bearing seeds in the preparation of protein foods for human consumption seems worthy of further study.

LITERATURE CITED

BRAMAN, W. W. 1931–32 The relative values of the proteins of linseed meal and cottonseed meal in the nutrition of growing rats. J. Nutrition, vol. 4, p. 249.

BURROUGHS, E. W., H. S. BURROUGHS AND H. H. MITCHELL 1940 The amino acids required for the complete replacement of endogenous losses in the adult rat. J. Nutrition, vol. 19, p. 363.

FINKS, A. J., AND CARL O. JOHNS 1921 Studies in nutrition. VI. The nutritive value of the proteins of the lima bean, Phaseolus lunatus. Am. J. Physiol., vol. 56, p. 205.

FISHER, R. A. 1928 Statistical methods for research workers. Edinburgh and London, 2nd ed. pp. 269. See page 107.

HAYWARD, J. W., H. STEENBOCK AND G. BOHSTEDT 1936 The effect of heat as used in the extraction of soy bean oil upon the nutritive value of the protein of soy bean oil meal. J. Nutrition, vol. 11, p. 219.

JOHNS, C. O., A. J. FINKS AND M. S. PAUL 1919 Studies in nutrition. I. The nutritive value of coconut globulin and coconut press cake. J. Biol. Chem., vol. 37, p. 497.

JONES, D. B., AND J. P. DIVINE 1944 The protein nutritional value of soybean, peanut, and cottonseed flours and their value as supplements to wheat flour. J. Nutrition, vol. 28, p. 41.

JONES, D. BREESE, AND JOSEPH C. MURPHY. With the assistance of Otto Moeller 1924 Cystine deficiency and vitamin content of the lentil, Lens esculenta moench. J. Biol. Chem., vol. 59, p. 243.

MAYNARD, L. A., AND F. M. FRONDA 1921 The relative growth-promoting value of the protein of coconut oil meal, and of combinations of it with protein from various other feeding stuffs. Cornell University, Mem. 50, p. 621.

MITCHELL, H. H. 1924 The biological value of proteins at different levels of intake. J. Biol. Chem., vol. 58, p. 905.

———— 1942 Biological methods of measuring the protein values of feeds. J. Animal Sci., vol. 2, p. 263.

———— 1944 The significance of the biological value of protein. Presented before the Division of Agricultural and Food Chemistry, American Chemical Society, at Cleveland, on April 6, 1944. To be published in Industrial and Engineering Chemistry.

MITCHELL, H. H., AND J. R. BEADLES 1944 Corn germ: A valuable protein food. Science, vol. 99, p. 129.

MITCHELL, H. H., E. W. BURROUGHS AND J. R. BEADLES 1936 The significance and accuracy of biological values of proteins computed from nitrogen metabolism data. J. Nutrition, vol. 11, p. 257.

MITCHELL, H. H., AND G. G. CARMAN 1924 The biological value for maintenance and growth of the proteins of whole wheat, eggs, and pork. J. Biol. Chem., vol. 60, p. 613.

———— 1926 The biological value of the nitrogen of mixtures of patent white flour and animal foods. J. Biol. Chem., vol. 68, p. 183.

MITCHELL, H. H., AND D. B. SMUTS 1932 The amino acid deficiencies of beef, wheat, corn, oats and soybeans for growth in the white rat. J. Biol. Chem., vol. 95, p. 263.

MITCHELL, H. H., AND V. VILLEGAS 1923 The nutritive value of the proteins of coconut meal, soy beans, rice bran and corn. J. Dairy Sci., vol. 6, p. 222.

MORGAN, AGNES FAY. With the cooperation of Florance B. King, Ruth E. Boyden, and V. Ann Petro 1931 The effect of heat upon the biological value of cereal proteins and casein. J. Biol. Chem., vol. 90, p. 771.

NEVENS, W. B. 1921 The proteins of cottonseed meal. II. Nutritive value. J. Dairy Sci., vol. 4, p. 552.

OLCOTT, H. S., AND T. D. FONTAINE 1941 The effect of autoclaving on the nutritive value of the proteins in cottonseed meal. J. Nutrition, vol. 22, p. 431.

SMUTS, D. B., AND A. I. MALAN 1938 Protein studies. Plant proteins. II. The biological values of lucerne meal sesamemeal, peanutmeal, coprameal, cottonseedmeal, and oatmeal. Onderstepoort J. Vet. Sci. and Animal Inds., vol. 10, p. 207.

STEWART, R. A., G. W. HENSLEY AND F. N. PETERS, JR. 1943 The nutritive value of protein. I. The effect of processing oat protein. J. Nutrition, vol. 26, p. 519.

STUDENT 1908 The probable error of a mean. Biometrika, vol. 6, p. 1.

WATERMAN, H. C., AND C. O. JOHNS 1921 Studies on the digestibility of proteins in vitro. I. The effect of cooking on the digestibility of phaseolin. J. Biol. Chem., vol. 46, p. 9.

WATERMAN, HENRY C., AND D. BREESE JONES 1921 Studies on the digestibility of proteins in vitro. II. The relative digestibility of various preparations of the proteins from the Chinese and Georgia velvet beans. J. Biol. Chem., vol. 47, p. 285.

ENRICHED, MORRIS TYPE AND WHOLE WHEAT FLOUR AS SOURCES OF THE B-COMPLEX VITAMINS [1]

BEULAH D. WESTERMAN AND E. G. BAYFIELD

Dept. of Food Economics and Nutrition, and Dept. of Milling Industry, Kansas Agricultural Experiment Station, Kansas State College, Manhattan

THREE FIGURES

(Received for publication August 21, 1944)

About one-third of the total calories of American and British diets is obtained from bread and cereals. For European countries other than Britain, this figure is increased to 50% or more. As flour occupies such an important place in the diet, it is desirable that it be of high nutritive value.

It is well known that the whole wheat grain contains more nutrients than patent flour. Several methods have been used to improve the nutritive value of patent flour. Addition of minerals and synthetic vitamins to patent flour, or modifying the milling process so as to retain more of the nutrients of the whole grain, are two means of increasing the mineral and vitamin content of flour. In addition bread may also be improved nutritionally by the use of high vitamin yeast and other supplements. Enrichment of flour is doubly important since it is used in many forms besides bread.

It was shown by Fairbanks ('39) that addition of milk solids to bread increased the nutritive value. Mitchell, Hamilton and Shields ('43) found that white bread enriched with thiamine, niacin, iron and riboflavin did not promote as good growth in rats as when non-fat milk solids were added. Chick ('40) reported that white flour of 73% extraction supplemented with casein, fat, salt mixture, fat-soluble vitamins and pure thiamine did not produce as good growth in rats as whole wheatmeal. Wright ('41) obtained the same rate of growth in animals with 85% wheatmeal and with white flour of 75% extraction fortified with thiamine.

Experiments with young college women by Free ('40) showed an improved state of nutrition when white bread made with high vitamin

[1] Contribution no. 123, Department of Home Economics.
Contribution no. 110, Department of Milling Industry.

yeast was ingested. Sealock, Basinski and Murlin ('41) found that the higher "indigestible residues" of whole wheat products did not interfere with the digestion and absorption of carbohydrate and fat. That the consumption of extra B-complex vitamins improves the biological value of proteins was shown by Murlin, Marshall and Kochakian ('41) when they tested the digestibility and biological value of whole wheat and white bread. Sealock and Livermore ('42) reported that peeled wheat bread was a good source of the B-complex vitamins and Alcock and Larmour ('42) were of the opinion that long extraction milling may solve the problem of more nutrients in flour.

Light and Frey ('43) obtained better weight gains when rats were fed white bread supplemented with lysine, valine, salt mixture, vitamins A, D, E, and riboflavin, than when fed either ordinary white bread or bread enriched at the old levels (previous to October, '43) and without the addition of riboflavin. Williams, Mason and Wilder ('43) made studies with human beings on diets low in thiamine and riboflavin. A supplement of enriched flour plus 6% milk solids gave the same results as whole grain flour. Lepkovsky ('44) in reviewing the bread problem states: "Unlike the English, they (the Americans) have not discussed with any degree of thoroughness the relative merits of the whole wheat bread, white bread and 'enriched' white bread."

For the purpose of gaining further information on this problem growth studies on albino rats were conducted in order to compare the relative values of whole wheat, with a Morris type of flour and enriched flour, as sources of the B-complex vitamins, when these are included as 30, 40 and 50% of the diet.

EXPERIMENTAL PROCEDURE

Young albino rats weighing between 40 to 50 gm. were used for the tests. Each animal was placed in an individual cage on a raised wire screen to prevent consumption of feces. Food and water were given ad libitum. The B-complex free basal diet consisted of vitamin free casein, 20%; cornstarch, 60%; corn oil, 12%; salt mixture, 5%; and cod liver oil, 3%. This was a modified Chase and Sherman ('31) diet. The casein supplied the amino acids that are lacking in wheat or flour, and the salt mixture the additional minerals needed. The cod liver oil furnished vitamins A and D. The rat does not require the addition of vitamins C and K; therefore the B-complex vitamins were the limiting factors.

All animals were fed the B-complex free diet for 12 days before the beginning of the test period in order partially to deplete their body

stores of these vitamins. At the end of the depletion period they were divided into groups of five or eight animals and placed on the test diets. The supplementing materials were whole wheat, Morris type of flour, patent flour enriched at the old levels with thiamine, niacin and iron, which will be designated as "old enriched flour" and patent flour enriched at the new or present levels with thiamine, niacin, riboflavin and iron, designated as "new enriched flour."

There were four series of tests. In the first series patent flour, whole wheat, old enriched, and Morris type flour were used as supplements. All these materials were obtained from one mill and one mill mix. The plain and enriched patent flour was of 70% extraction. The whole wheat was finely ground in a burr mill. In order to test wheat and flour from different sources, another mill supplied the flour and wheat for the second and third series of tests, while still another supplied the materials for the fourth series.

The average American diet contains approximately 30% cereals. In some cases this may be increased to 40 or 50%; therefore the supplements were incorporated into the diets at 30, 40 and 50% levels. They replaced an equivalent amount of cornstarch in the basic diet. Weights of the animals were recorded every 6 days. The average weights of the animals on each test material are shown by growth curves.

RESULTS

The growth curves show the average weights of the five animals with each supplement at a 30% level (fig. 1). The supplements were whole wheat, Morris type flour and old enriched flour. One group of animals was left on the B-complex free diet. These served as negative controls and showed that the basal diet would not support growth as these animals lost weight and died before the twenty-fourth day.

The animals on the Morris type flour and the old enriched flour showed parallel weight changes and lost an average of 5 gm. during the 48-day test. Those receiving whole wheat gained an average of 11 gm. showing that when fed at a level of 30% of the diet, whole wheat used as a source of the B-complex vitamins, promoted better growth than either the Morris type flour or old enriched flour. They also were more alert and their fur had a better texture.

The animals on whole wheat at the 50% level (fig. 1) gained an average of 41 gm. during the 48-day test period. They had the appearance of healthy rats. When ordinary patent flour was included at this level, the animals lost weight slowly and died by the thirty-sixth day. Those on the old enriched flour weighed on the average the same at the end

of the experiment as at the beginning, while those on the Morris type flour made an average gain of 6 gm. and had a better appearance than those on the old enriched flour.

The different materials were analyzed for thiamine content. The wheat from which the Morris type flour was milled contained 5.3 μg. per gram, the Morris type flour contained 3.3 μg. per gram, while the old enriched flour assayed 3.97 μg. per gram. Although the old enriched flour contained more thiamine than the Morris type flour, the animals made slightly better gains on the latter, when both were fed at the 50% level in the diet, showing that other factors besides thiamine are involved.

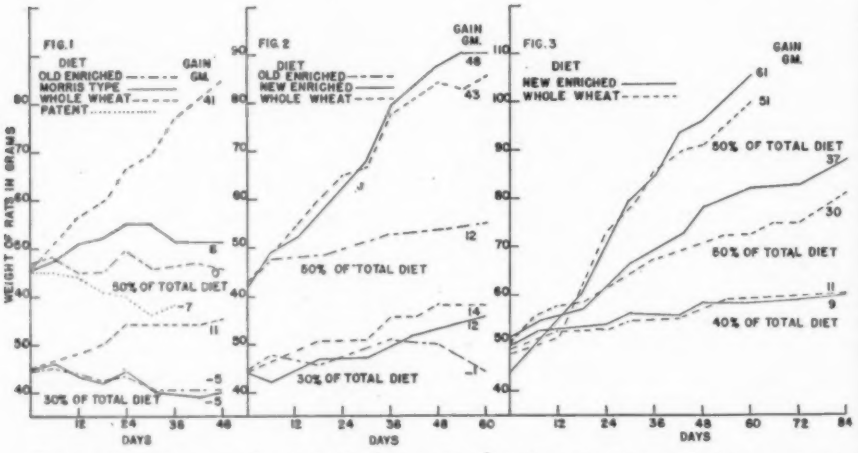


Fig. 1 Growth curves of rats in the first series of tests using flour and wheat as sources of the B-complex vitamins.

Fig. 2 Growth curves of rats in the second series of tests.

Fig. 3 Curves showing the average rate of growth of the rats. The four lower curves show the third test and the two upper curves the fourth test.

A second series of experiments was conducted in the same manner, except that new enriched flour was included. At the 30% level (fig. 2) the animals on the old enriched flour gained on the average 5 gm. during the first 48 days of the test but lost this gain before the sixtieth day. Throughout the test, those on the whole wheat maintained a slightly higher weight gain than those on the new enriched flour. At the end of the 60 days those on the new enriched flour had gained 12 gm. and those on whole wheat 14 gm.

At the 50% levels (fig. 2) the animals on the old enriched flour gained an average of 12 gm. during the entire period while those on whole

wheat and new enriched flour made almost parallel gains until the thirty-sixth day, after which those on the new enriched flour made better gains. At the end of 60 days those on whole wheat had made an average gain of 43 gm. and those on new enriched flour had gained 48 gm.

It was decided to run a third series using the same flour and whole wheat as in the second series but with the supplements at the 40 and 50% levels and including a group of animals on the stock diet. This was an 84-day test and eight animals were used in each group (fig. 3). The average weight gains, when the wheat and new enriched flour were fed at the 40% levels, were practically parallel throughout the test. At the 50% level, the curves were parallel for the first 36 days, but by the eighty-fourth day the animals on the new enriched flour had gained an average of 7 gm. more than those on whole wheat. None of these animals grew as well as those on the stock diet. The total average gain of the stock animals during the 84 days was 202 gm.

At the end of the experiment some of the animals on each supplement were autopsied. No excess fat was found in the livers. As compared to the animals on the stock diet, the animals on the test diets had normal organs except for size.

The thiamine content of the whole wheat was 3.58 μg. per gram, for old enriched flour 3.83 μg. per gram, and 3.96 μg. per gram for new enriched flour. The riboflavin values were 1.14 μg. per gram for whole wheat as compared to 0.41 μg. per gram for old enriched flour and 2.56 μg. per gram for new enriched flour. The greatest difference is shown in the riboflavin values. As the new enriched flour contained more riboflavin than either the whole wheat or old enriched flour the use of riboflavin along with thiamine may account for the increased growth of the animals on the new enriched flour. Higgins, Williams and Mason ('43) fed rats a diet low in thiamine, analogous to that consumed by some humans and reported that increasing the thiamine in the bread did not promote better growth but the addition of both thiamine and riboflavin did induce a growth rate equal to that of whole wheat bread.

Even though the growth rate of the rats may have been improved by the addition of riboflavin as well as thiamine and niacin in the new enriched flour, the maximum growth rate thus attained did not equal that of the animals fed the stock diet. The stock diet consisted of a commercial dog food [2] and contained an adequate amount of all the B-complex vitamins. Neither whole wheat or enriched flour contained enough of all of the B-complex vitamins to produce optimal growth, but the addition of thiamine, niacin and riboflavin definitely improved

[2] Purina Dog Chow.

the nutritive value of patent flour sufficiently to make it equal to or better than whole wheat in promoting growth, depending on the level fed.

Wheat and flour obtained from a different mill were used in another series of tests in order to eliminate any errors due to milling processes or mixing of the enriching materials or other factors. Again the flour was milled from the same lot of wheat as was used in the test. The new enriched flour and the wheat were fed at the 50% level in the diet. Five animals were used on each test. The growth curves were parallel for 36 days of the test (fig. 3) at which time the rats on the new enriched flour began to gain at a faster rate than those on whole wheat. At the end of the 60-day period the animals on the new enriched flour had made an average gain of 61 gm. while those on whole wheat had achieved an average gain of 51 gm.

The wheat assayed 4.10 μg. per gram for thiamine, 1.08 μg. per gram for riboflavin and the new enriched flour 4.69 μg. and 3.13 μg. per gram respectively. In this case the enriched flour was higher in both thiamine and riboflavin content than the whole wheat which may be the reason for the difference of 10 gm. in the weight gains of the two groups as compared to a difference of 7 gm. in the first test at the 50% level and 5 gm. in the second test. These differences in the average growth rates between the animals fed whole wheat and new enriched flour seem to be rather definite evidence that flour, enriched at the new levels and with riboflavin, is a better source of the B-complex vitamins than whole wheat when these are fed as 50% of the total diet.

SUMMARY AND CONCLUSIONS

Four series of experiments were conducted on albino rats to compare the relative growth promoting values of whole wheat, Morris type flour, patent flour enriched at the old levels, and patent flour enriched at the new levels, as a source of the B-complex vitamins. The first experiment included whole wheat, Morris type flour and old enriched flour, the second, compared whole wheat, old enriched flour and new enriched flour at 30 and 50% levels in the diet, while the third made comparisons at the 40% level. A fourth test was run on wheat and flour from another mill at the 50% level.

Under the conditions of these experiments, the results indicate that whole wheat is a better source of the B-complex vitamins than either Morris type flour or patent flour, enriched at the old levels, when these materials make up 30 or 50% of the diet. At a 30% level whole wheat is slightly better than patent flour, which has been enriched at the new

levels. Whole wheat and new enriched flour promote the same amount of growth when fed at a 40% level, while at a 50% level the new enriched flour is better as a source of the B-complex vitamins than whole wheat.

LITERATURE CITED

ALCOCK, A. W., AND R. K. LARMOUR 1942 High vitamin flours. Canad. Chem. and Process Ind., vol. 26, p. 3.

CHASE, E. F., AND H. C. SHERMAN 1931 A study of the determination of the antineuritic vitamin B. J. Am. Chem. Soc., vol. 53, p. 3506.

CHICK, H. 1940 Nutritive value of white flour with vitamin B_1 added and of wholemeal flour. Lancet, vol. 239, p. 511.

FAIRBANKS, B. W. 1939 A study by the paired feeding method of the nutritive value of bread made with milk solids. Cereal Chem., vol. 16, p. 404.

FREE, A. H. 1940 Increase of vitamin B intake by the use of special high vitamin bread. Cereal Chem., vol. 17, p. 725.

HIGGINS, G. M., R. D. WILLIAMS AND H. L. MASON 1943 Results of feeding rats a thiamine-low diet of a type consumed by human beings. J. Nutrition, vol. 25, p. 229.

LEPKOVSKY, SAMUEL 1944 The bread problem in war and in peace. Physiol. Rev., vol. 24, p. 239.

LIGHT, R. F., AND C. N. FREY 1943 The nutritive value of white and whole wheat breads. Cereal Chem., vol. 20, p. 645.

MITCHELL, H. H., T. S. HAMILTON AND J. B. SHIELDS 1943 The contribution of non-fat milk solids to the nutritive value of wheat breads. J. Nutrition, vol. 25, p. 585.

MURLIN, J. R., M. E. MARSHALL AND C. D. KOCHAKIAN 1941 Digestibility and biological value of whole wheat breads as compared with white breads. J. Nutrition, vol. 22, p. 573.

SEALOCK, R. R., D. K. BASINSKI AND J. R. MURLIN 1941 Apparent digestibility of carbohydrate, fat and "indigestible residue" in whole wheat and white breads. J. Nutrition, vol. 22, p. 589.

SEALOCK, R. R., AND A. H. LIVERMORE 1942 The vitamin B complex of peeled wheat bread. J. Nutrition, vol. 25, p. 265.

WILLIAMS, R. D., H. L. MASON AND R. M. WILDER 1943 Evaluation of the nutritive contributions of enriched white flour. J. Am. Med. Assn., vol. 121, p. 943.

WRIGHT, M. D. 1941 The nutritive value of bread. Fortified white flour and national wheatmeal compared. Brit. Med. J., vol. 2, p. 689.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THIAMINE REQUIREMENT OF THE RAT

O. L. KLINE, LEO FRIEDMAN AND E. M. NELSON
Vitamin Division, U. S. Food and Drug Administration, Washington, D. C.

ONE FIGURE

(Received for publication August 14, 1944)

The relation in animals of thiamine requirement to carbohydrate metabolism has been extensively studied, and has been discussed in detail by Ochoa ('42). The effect of increased metabolic rate on the need for thiamine, also well known, is pointed out by Cowgill ('39) and Williams and Spies ('38). Environmental temperature, however, in relation to thiamine utilization has received relatively little attention. It may be expected that any environmental factor that influences energy utilization or output will in turn affect the requirement for thiamine. In our work on biological methods for determining thiamine a profound effect of environmental temperature on thiamine requirement has been demonstrated both in rat-curative and rat-growth experiments. It has been found that careful temperature control is essential in obtaining a high degree of accuracy, particularly in the rat-curative type of assay. In these studies the cure of polyneuritis in rats and the growth of rats receiving varying levels of thiamine were used as criteria of thiamine requirement. The animals were kept in air-conditioned rooms with adequate temperature and humidity control.

Studies involving polyneuritis

The rat-curative studies were conducted in accordance with the U.S.P. method of assay for thiamine [1] because there is convincing evidence that the duration of the cure of polyneuritis with the diet prescribed is not influenced by dietary factors other than thiamine. Litters of white rats at the age of 13 days were transferred with their mothers to the U.S.P. thiamine-deficient diet, and were later weaned at a body weight of 40 to 50 gm. The thiamine-deficient diet was fed ad libitum and the animals were kept in cages with wire screen bottoms

[1] The Second Supplement to the Pharmacopoeia of the United States of America, Eleventh Decennial Revision, p. 129.

throughout the experiment. Each litter of rats was kept in one cage until the twenty-fifth day of the depletion period and the animals were then segregated into individual cages. The animals were weighed weekly and after the twenty-fifth day of the depletion period were observed twice daily for symptoms of polyneuritis. At the first and at each successive occurrence of polyneuritis the animals were given a single dose of the U.S.P. Thiamine Hydrochloride Reference Standard by stomach tube. The animals were maintained during the curative periods at a temperature of 78°, 85°, or 90°F., and were transferred from one temperature to another at the time polyneuritis occurred and a dose of thiamine was administered. Size of doses, environmental temperatures, and the resulting curative responses measured in days are given in tables 1 and 2.

TABLE 1

Duration of curative response to 6-μg. doses of thiamine at different environmental temperatures.

RAT NO.	FIRST PERIOD 78° F.	SECOND PERIOD 85° F.	RAT NO.	FIRST PERIOD 78° F.	SECOND PERIOD 85° F.
	days	*days*		*days*	*days*
3534	6	10	3548	10	10
3536	5	11	3524	11	15
3523	9	9	3532	8	10
3501	12	15	Average	8.7	12.4
3503	12	16	$t = \frac{\text{difference between averages}}{\text{standard error of difference}} = 3.096$		
3542	5	15			
3527	9	13	therefore $P<0.01$		

In a preliminary study involving only four animals kept alternately at 78° and 90° F. over four curative periods it was found that a 6-μg. dose of thiamine produced curative responses of 6 to 9 days duration at 78° F. and 11 to 15 days at 90° F. The duration of the cure did not seem to be influenced by the temperature at which the animal had been kept prior to the administration of the curative dose.

The data presented in table 1 are for ten animals maintained during the first curative period at 78° F. and during the second at 85° F. Although two of the animals had curative periods of equal length at the two temperatures, the averages for the groups are appreciably different. As statistically tested by use of Student's "t" test, the difference between the averages is very significant. The difference in the curative periods at the two temperatures is sufficient to indicate a difference of approximately 20% in dose levels under normal test conditions.

In another experiment ten animals were carried through four successive curative periods, maintained at 78° F. during the first, at 90° F. during the second, and at 78° F. for the third and fourth periods. Doses of 6 μg. of thiamine were administered for the first and third periods while 3-μg. doses were given the animals at the beginning of their second and fourth periods. In table 2 it will be seen that the average lengths of the first and the third curative periods induced by 6 μg. are quite similar, 9.1 and 9.9 days, respectively, while the average length of the second period, the response to a 3-μg. dose of thiamine in the 90° F. room, is 11.6 or greater than that of the first or third periods. In the fourth period where the animals were kept at 78° F. and received 3-μg.

TABLE 2

Successive curative periods with different environmental temperatures and thiamine dosages.

RAT NO.	FIRST PERIOD 78° F. 6γ B_1	SECOND PERIOD 90 °F. 3γ B_1	THIRD PERIOD 78° F. 6γ B_1	FOURTH PERIOD 78° F. 3γ B_1
	days	*days*	*days*	*days*
3936	7	11	7	3
3938	7	11	8	No cure
3973	7	14	8	4
3985	14	13	19	3
3987	11	11	7	2
3988	12	14	11	No cure
4016	10	15	15	No cure
4040	7	9	5	2
4042	7	7	9	No cure
4043-A	9	11	10	3
Average	9.1	11.6	9.9	

doses there were no cures of polyneuritis in four animals and the duration of cure was from 2 to 4 days for the others. This response is typical for this dose and does not reflect a failure of the rat to respond owing to repeated occurrence of polyneuritis. Since it has been adequately demonstrated that at a given temperature, over a wide range of thiamine dosage, the duration of curative response is proportional to the dose administered (Kline, Tolle and Nelson, '38), and since the duration of cure with 3 μg. at 90° F. is greater than with 6 μg. at 78° F. the data presented are interpreted to indicate a 50% reduction in thiamine requirement for the rat under the environmental conditions described.

Studies involving rat growth

The animals used for the growth study were weaned from mothers on an adequate stock diet, at a weight of 40 to 50 gm. and were fed

the U.S.P. thiamine-deficient diet ad libitum. They were kept at 78° F. in litter cages on wire-screen bottoms until records of body weight, taken as specified in the A.O.A.C. procedure (Kline, Hall and Morgan, '41) for the growth assay of thiamine, indicated the end of the depletion period. This condition is reached on the day when the body weight of the animal is equal to or less than that on the fifth preceding day. The animals were then placed in individual cages, grouped as desired, and records of body weight and food consumption were taken twice a week. In all instances where thiamine intake was controlled, the desired dose of the U.S.P. Thiamine Hydrochloride Reference Standard was administered daily by stomach tube.

In a preliminary study two groups of twelve animals that had been depleted were maintained at 78° and 90° F. and fed 2.5 μg. of thiamine daily for 33 days. The dose was then increased to 10 μg. daily for 10

TABLE 3

Weight gains and food consumption of rats at indicated temperatures and thiamine intakes.

DAILY SUPPLEMENT	AVERAGE DAILY WEIGHT GAINS		AVERAGE DAILY FOOD INTAKE	
	78° F.	90° F.	78° F.	90° F.
μg.	*gm.*	*gm.*	*gm.*	*gm.*
2.5 (33 days)	0.7	1.0	7.0	6.0
10 (10 days)	4.0	3.1	13.3	8.6
100 (7 days)	4.1	2.6	14.0	8.5
None — Both groups at 78°F. (7 days)	3.0	2.7	14.0	10.7

days and further to 100 μg. daily for 7 days. Both groups were then kept for 7 days at 78° F. with no supplement. Results are given in table 3. Greater gains in weight were made during the 33-day period by the animals at the higher temperature, even though their food consumption was lower. With an increase in thiamine intake to a near optimum level or to a level ten times the optimum, this situation was reversed, and gain in weight and food intake were greater at 78° F. During the last period when both groups of animals were maintained at 78° F. without thiamine, the food consumption of those removed from the higher temperature increased markedly.

In another study forty-eight depleted animals were divided into six comparable groups, three of which were kept in the room maintained at 78° F. and the remaining three groups transferred to a room maintained at 90° F. One group in each room was fed 2.5 μg. of thiamine daily; a second group in each room received 10 μg. daily; and the remaining

groups were changed to the diet that is used for the laboratory stock colony. Records were taken twice weekly of body weight and food consumption for a period of 5 weeks for all animals, and for 10 weeks for the groups receiving the lower level of thiamine. Results of the 5-week experimental period are given in table 4, and the growth rates of the two groups that received the lower level of thiamine for 10 weeks are illustrated in figure 1.

Rates of growth are expressed as average daily weight gains in grams — for simplicity of comparison, and also in conjunction with average daily food intake, to allow convenient calculation of the amount of gain per gram of food consumed. In the groups that received 2.5 μg. of thiamine daily, a suboptimal level, the animals at the higher temperature were able to make greater gains with lower food intakes, and this occurred during a period of uniform weight increase, before there was evidence of loss of weight in any individual, or of occurrence of polyneuritis. The superiority of utilization of food for growth is reflected in the grams gain per gram of food intake, the difference for these two groups being 0.072 gm. in favor of the animals at the higher temperature. For those animals that received 10 μg. of thiamine per day, the greater growth occurred in the lower temperature, since thiamine no longer was the limiting factor. Owing to the reduced food intake at the higher temperature, however, the amount of gain for each gram of food consumed was still in favor of the higher temperature, with a difference of 0.056 gm. Although growth on the diet of natural foodstuffs was improved, the relationships were much the same as for the intermediate groups, and the difference of gain per unit of food intake was 0.060 gm.

TABLE 4

Comparison of weight gains and food consumption of rats at indicated temperatures and thiamine levels.

(Experimental period — 35 days)

	B_1 DEFICIENT DIET PLUS 2.5 MICROGRAMS B_1 DAILY		B_1 DEFICIENT DIET PLUS 10 MICROGRAMS B_1 DAILY		STOCK DIET WITH NO ADDED B_1	
	78° F.	90° F.	78° F.	90° F.	78° F.	90° F.
Av. daily weight gains (gm.)	0.8	1.1	3.5	3.0	5.1	4.6
Av. daily food intake (gm.)	7.0	5.6	12.7	9.0	19.0	12.6
Grams gain per gram food intake	0.124	0.196	0.275	0.333	0.304	0.364

In the two groups of animals given the lower level of thiamine and maintained throughout a 10-week period growth increased uniformly at the higher temperature with no evidence of polyneuritis. For the animals maintained at 78°F., the growth curve shows a plateau after the seventh week, and four of the eight animals died of polyneuritis before the end of the experimental period.

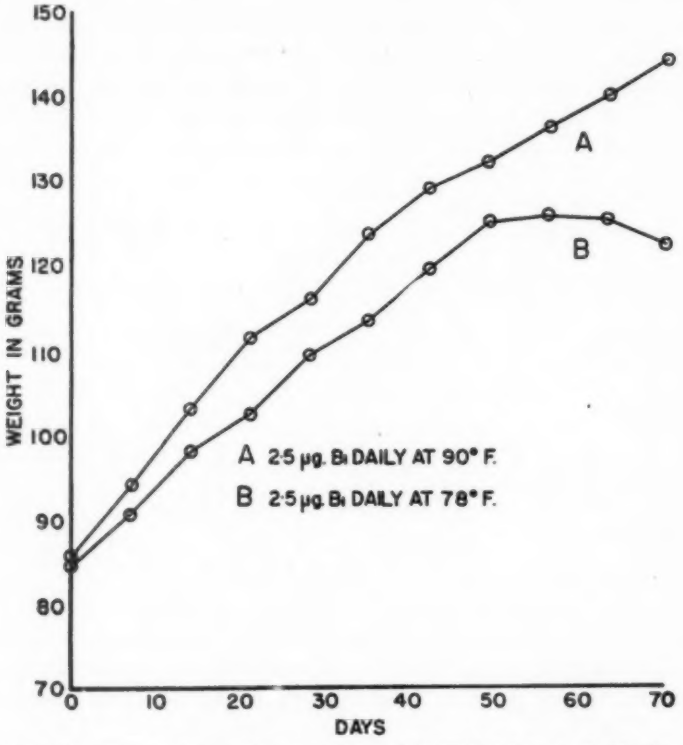


Fig. 1 Growth curves on groups of rats at 78° and 90°F. fed 2.5 μg. of thiamine daily.

DISCUSSION

From results of the rat-curative experiments presented it would seem logical to conclude that as environmental temperature of the rat is increased, the daily requirement for thiamine is reduced. Such a conclusion is in keeping with what is known about the function of thiamine in the utilization of food for energy. With increased environmental temperature the amount of energy needed for maintenance of body temperature is decreased. Drill and Shaffer ('42) have concluded that

the increase in energy expended in experimental hyperthyroidism caused an increased requirement for vitamin B_1 and other vitamins of yeast.

In the growth studies described, thiamine intake and environmental temperature were the two important factors affecting growth response and food consumption. In the animals maintained at the higher environmental temperature consumption of food was lower than that of control animals, owing undoubtedly to a reduced metabolic rate. A depressing effect of increased environmental temperature upon metabolic rate has been pointed out by Horst, Mendel and Benedict ('30) and has been discussed more recently by Herrington ('40). A direct relationship between growth rate and rate of metabolism has been demonstrated by Kibler and Brody ('42). Under the conditions of our experiments with optimal thiamine intake food consumption and growth response were directly affected by environmental temperature, and thus could not be related to thiamine requirement. For a clear demonstration of the effect of temperature upon thiamine requirement it is essential to use a suboptimal thiamine level, where the animal is sensitive to variations in factors affecting thiamine requirement. Sarrett and Perlzweig ('43) although obtaining, with paired feeding technic, higher growth rates in rats at elevated temperatures, were unable to demonstrate an effect of environmental temperature on thiamine requirement owing undoubtedly to the feeding of adequate levels of thiamine.

Mills ('41) has reached the conclusion from studies similar to ours that thiamine requirement of the rat is increased with increased environmental temperature. It should be pointed out that in his experiments, since thiamine supplements were mixed with the diet, thiamine intake for each animal varied with food intake, and was, in effect, controlled by environmental temperature. If actual amounts of thiamine ingested are calculated for these groups of animals that received suboptimal intakes of thiamine, it will be noted that the animals kept at the higher temperature made greater weight gains on less thiamine than the animals at the lower temperature. This relationship is similar to that found in our experiments in which thiamine, fed as a separate supplement, was independent of food intake.

It is to be expected that a diet, adequate in respect to thiamine, when consumed in a temperate climate, would also be satisfactory under tropical conditions, even though a reduced food intake resulted from the higher environmental temperature.

In experiments similar to those reported here, Edison and Molitor ('43) have concluded that thiamine requirements in the tropics are not

greater than in temperate climates, but on the contrary are lower, owing to decreased activity of the animal.

SUMMARY

Evidence has been obtained in studies on rats involving both cure of polyneuritis and growth, which shows that an increase in environmental temperature results in a decreased thiamine requirement. It appears that this decrease approximates the decrease in caloric requirement at the elevated temperature. Maintenance of a uniform environmental temperature is essential for precision in performing the rat-curative assay for thiamine.

ACKNOWLEDGMENT

The authors wish to thank Lila F. Knudsen for her helpful suggestions and statistical analysis.

LITERATURE CITED

COWGILL, G. R. 1939 Human requirements for thiamine. The Vitamins. American Medical Association, Chicago, Ill., pp. 243–244.

DRILL, V. A., AND C. B. SHAFFER 1942 Effect of vitamin B_1 and yeast on calorie intake and weight balance of hyperthyroid dogs. Endocrin., vol. 31, p. 567.

EDISON, O. A., AND H. MOLITOR 1943 Private Communication.

HERRINGTON, L. P. 1940 The heat regulation of small laboratory animals at various environmental temperatures. Am. J. Physiol., vol. 129, p. 123.

HORST, K., L. B. MENDEL AND F. G. BENEDICT 1930 The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. J. Nutrition, vol. 3, p. 177.

KIBLER, H. H., AND S. BRODY 1942 Metabolism and growth rate of rats. J. Nutrition, vol. 24, p. 461.

KLINE, O. L., W. L. HALL AND J. F. MORGAN 1941 An improved rat-growth method for the assay of vitamin B_1 including sulfite treatment of dietary constituents. J. Assoc. Official Agr. Chemists, vol. 24, p. 147.

KLINE, O. L., C. D. TOLLE AND E. M. NELSON 1938 Vitamin B_1 assay by a rat-curative procedure. J. Assoc. Official Agr. Chemists, vol. 21, p. 305.

MILLS, C. A. 1941 Environmental temperatures and thiamine requirements. Am. J. Physiol., vol. 133, p. 525.

OCHOA, S. 1942 The Biological Action of the Vitamins. University Chicago Press, Chicago, Ill., pp. 17–41.

SARRETT, H. P., AND W. A. PERLZWEIG 1943 The effect of high temperature and B-vitamin levels of the diet upon the metabolism and body composition of rats. J. Nutrition, vol. 26, p. 611.

WILLIAMS, R. R., AND T. SPIES 1938 Vitamin B_1 in medicine. The Macmillan Company, New York, pp. 261–265.

THE RELATION OF THE DIETARY CA:P RATIO TO SERUM CA AND TO PARATHYROID VOLUME

HERBERT C. STOERK AND WILLIAM H. CARNES
WITH THE ASSISTANCE OF CLAUDIA FRENCH AND THELMA STOUT
Department of Pathology, Columbia University College of Physicians and Surgeons, New York

THREE FIGURES

(Received for publication August 21, 1944)

It has been shown by a number of investigations (Ham et al., '40; Carnes et al., '42) that various conditions associated with hypocalcemia or hyperphosphatemia, or both, lead to hyperplasia of the parathyroid glands. Evidence has also been given that parathyroid enlargement in such instances may be associated with increased secretory activity of the glands (Baumann and Sprinson, '39). The changes in the levels of blood Ca and P in these instances are usually reciprocal and the question has arisen whether the parathyroid enlargement is primarily related to the lowering of the blood Ca or to the increase in blood PO_4.

Ham and coworkers ('40), by adding phosphate to a stock diet, were able to obtain a small increase in the serum PO_4 of rats without changing the serum Ca. The parathyroids of these animals failed to enlarge. The glands did enlarge in rats on a Steenbock low Ca diet with low serum Ca values. It was concluded that hypocalcemia and not hyperphosphatemia is the stimulus to parathyroid enlargement.

In contrast to this, on adding graduated amounts of P to the Steenbock low P diet, Carnes and coworkers ('42) obtained stepwise increases in parathyroid volume in younger rats associated with rising serum PO_4 levels even in the absence of hypocalcemia. However at both extremes of the scale of dietary Ca/P ratio, the serum calcium changes were significant and the serum PO_4 values not always consistently altered. Further experience has cast doubt on the reliability of data obtained with the Steenbock diet which is inadequate in several respects and produces a very poor general condition of the experimental animals. Differences in the basal diet, form and quantity of added PO_4, and age of the experimental animals may have contributed to the discrepancies between the results of these two groups.

Recently Patt and Luckhardt ('42) concluded from a series of experiments on dogs that a low blood calcium is a direct stimulus for the parathyroid glands to produce more hormone. They lowered the blood calcium in their animals by injecting oxalate and also perfused decalcified blood through thyroid-parathyroid preparations, obtaining what they believed to be evidence for increased parathyroid activity. Possible alterations in blood PO_4, brought about by the procedures, were not excluded in these experiments.

The changes in Ca and PO_4 levels of the blood in response to variations in dietary Ca and P have been thoroughly studied by Shohl and Wolbach ('36) in an investigation of rickets in rats. They confirmed the findings of Kramer and Howland ('32) and of Bethke et al. ('32) that the blood levels of Ca and PO_4 reflect the dietary Ca/P ratio. Lowering this ratio decreased the serum Ca and increased the serum PO_4. Raising the ratio had the opposite effect on the serum values. They also found that, with a constant dietary Ca/P ratio, raising the absolute amounts of Ca and P in the diet produced a corresponding rise in both the serum Ca and serum PO_4.

If the blood level of either Ca or PO_4 is the primary stimulus of the parathyroid gland, it should be possible to determine which of these two factors is the essential one by experimentally altering the dietary intake of Ca and P independently over a wide range and by following the consequent changes in the blood levels and parathyroid volume. This can be done with a minimum of interference with the general nutrition in adult rats on a good basal ration.

EXPERIMENTS

Male albino rats, in most instances litter-mates, weaned at the age of 4 weeks and placed on our stock diet [1] were selected from the stock colony at 10 weeks of age. They were fed for 4 weeks a diet [2] devised by Zucker and Berg ('43) which is adequate in every essential except Ca and P and is thoroughly freed of vitamin D. This diet favors optimal growth (Zucker and Zucker, '42) if supplemented with proper amounts and Ca and P. The desired amounts of Ca and P were added in the form of $CaCO_3$ and KH_2PO_4. After 4 weeks the animals were anesthetized with ether and blood was drawn from the right auricle. Individual analyses were made for Ca (Clark and Collip, '25) and inorganic

[1] Rockland rat diet (D-free).

[2] Heated egg albumen (E) or alcohol extracted beef fibrin (F), 20%; modified Wesson salt mixture, 1.2%; Wesson oil, containing 5% carotene in oil, 2.0%; rice bran extract, 10%; cane sugar, to make 100%.

P (Kuttner and Cohen, '27) on fresh samples of serum. The parathyroid glands were fixed in Bouin's fluid, imbedded in paraffin, serially sectioned at 10 micra, and the volumes determined by tracings and planimeter measurements. Bones were sectioned and studied histologically and the bone ash was determined on the right femur in some of the groups.[3] Serum protein estimations were made in some groups by the falling drop method.

TABLE 1

Chemical and anatomical data on 14-week-old rats following 4-week feeding of diets of differing Ca and P content.

GROUP	DIET	% DIETARY		DIETARY CA/P RATIO	BODY WEIGHT (GM.)		PARATHYROID VOLUME (MM.³)	SERUM		SERUM CA/PO_4 RATIO	BONE ASH %	NO. RATS
		CA	P		Initial	Final		CA (MG. %)	PO_4			
1	F–908–A	1.053	.07	15.1	197	246	.173	11.9	3.4	3.5	60.2[1]	10
2	E–908	.615	.07	8.8	194	225	.181	11.6	2.5	4.6	..[1]	5
3	F–918–A	2.053	1.17	1.8	163	239	.311	10.3	7.3	1.4	..	9
4	F–918	1.053	.62	1.7	164	248	.313	10.4	8.2	1.3	..	8
5	F–918	1.053	.62	1.7	201	269	.283	11.1	7.1	1.6	64.0	6
6	E–918	1.015	.62	1.6	199	251	.348	10.6	..	..	62.4	6
7	E–907	.615	.47	1.3	178	241	.369	11.0	..	..	63.1	5
8	F–913	.053	.07	.8	207	269	.371	9.6	4.6	2.1	61.0[1]	6
9	F–909	.053	.47	.11	202	252	.475	8.1	7.3	1.1	56.0[1]	5
10	E–909	.015	.47	.03	178	188	.508	7.7	..	..	59.2[1]	6
11	E–909	.015	.47	.03	212	223	.551	7.3	...	..	..	6

[1] Rickets present.

RESULTS AND DISCUSSION

The complete data are given in table 1. By adding either Ca or P, or both simultaneously to the basic diet, nine variations of the dietary Ca/P ratio were obtained ranging from 15 to 0.032. The absolute level of dietary Ca varied from approximately 2% to 0.015% and the P from approximately 1.2% to 0.07%. Because of the age of the animals and the relatively short duration of the experiment, growth was only moderately retarded on low Ca and low P variations of the diet. Both types of rickets were mild as judged histologically and by the bone ash values.

On the basis of parathyroid size the animals fall into three distinct divisions. The glands were very small when the dietary Ca/P ratio was far above optimal (groups 1 and 2). The glands were very large when this ratio was far below optimal (groups 9–11). When the dietary

[3] We are indebted to Miss Margaret Young for the bone ash determination.

Ca/P ratio approached the optimal range (9), even though the absolute quantities of Ca and P were varied over an extremely wide range, the glands were intermediate in size and the groups did not differ significantly between each other (groups 3–8). Figure 1 illustrates the interpendence of parathyroid volume and the per cent of dietary Ca and P. Figure 2a shows, incidentally, that the relationship between parathyroid volume and the logarithm of the dietary Ca/P ratio closely approximates a straight line.

The effect of the diets on serum Ca level was inverse to their effect on the parathyroid volume. The table shows that on the basis of serum values the animals fall into the same three divisions that were made on the basis of parathyroid size. On a diet of high Ca/P ratio there was a mild hypercalcemia (groups 1 and 2). On a diet of very low Ca/P ratio there was a moderate hypocalcemia (groups 9–11). On a diet with a Ca/P ratio approaching the optimal, the serum Ca fell within the accepted range of normal, even though the absolute quantity of dietary Ca was varied within very wide limits (groups 3–8). Figure 2b shows that the relationship between the serum Ca level and the logarithm of the dietary Ca/P ratio also approximates a straight line. Under the conditions of our experiment the serum Ca level was not proportional to the per cent of dietary Ca at a constant Ca/P ratio. The serum Ca was not significantly different on diets of 2, 1, and 0.6% Ca, respectively (groups 3–7) and it was not greatly depressed with as little as 0.053% dietary Ca (group 8) when the dietary Ca/P ratio remained in the neighborhood of 1. These findings are confirmed by unpublished data on a sufficient number of animals which at weaning (age 4 weeks) were placed on the experimental diets for 4 weeks and then killed.

The serum phosphate level has been found, in agreement with Shohl and Wolbach ('36), to depend on both the dietary Ca/P ratio and the absolute quantity of dietary P up to a certain maximal level of serum PO_4. This maximum is apparently dependent upon factors other than dietary Ca and P and is also a function of the age of the animal. It was approximately 7–8 mg. % in this experiment but in immature rats it is about 10–12 mg. %. The level of serum PO_4 thus does not bear a simple inverse relationship to the serum Ca level.

Figure 3 shows that there is a fairly close inverse proportionality between the parathyroid size and the level of serum Ca over the entire range investigated. This relationship deviates somewhat from a straight line, perhaps due to an inherent limitation of maximal para-

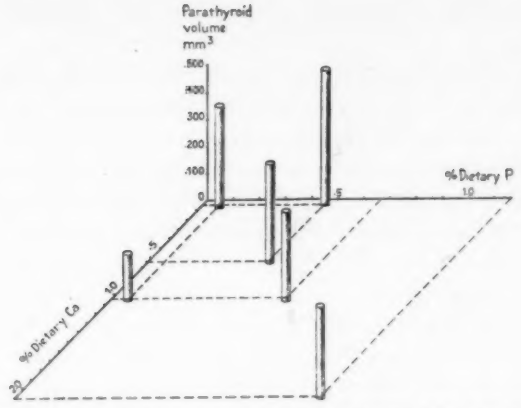


Fig. 1 Relationship between the per cent dietary calcium, the per cent dietary phosphorus, and the parathyroid volume.

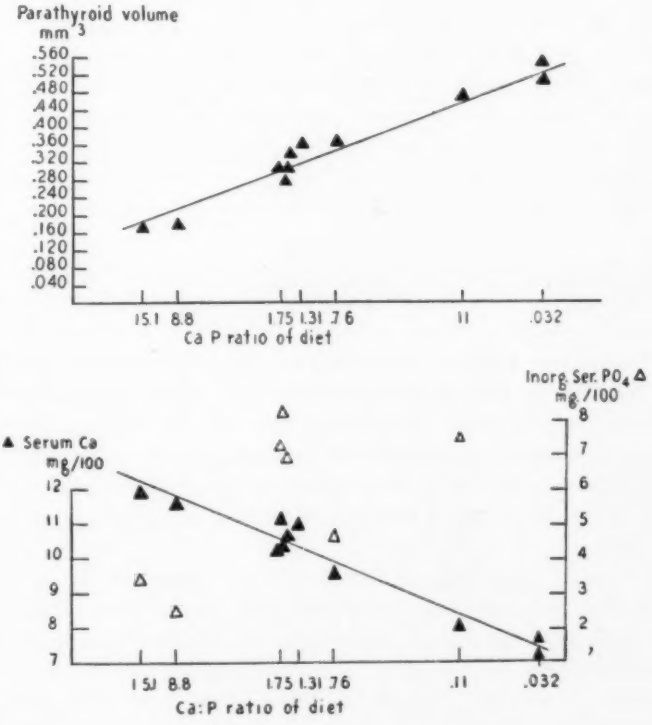


Fig. 2a Relationship between the parathyroid volume and the logarithm of the Ca/P ratio of the diet.

Fig. 2b Relationship between the serum calcium concentration and the logarithm of the Ca/P ratio of the diet.

thyroid growth in the hypocalcemic groups within the period of 4 weeks. The same deviation in the line could be due to a limitation, by factors other than the diet, in the maximal serum Ca attainable. The poorest alignment of the points occurs in the midportion of the curve within the normal range of serum Ca. This scattering of the points is not eliminated by expressing the parathyroid volume per unit of body weight.

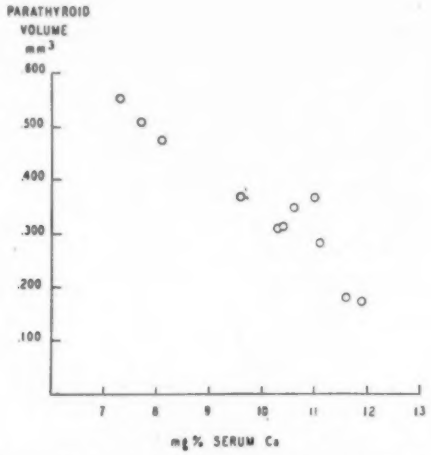


Fig. 3 Relationship between the serum calcium concentration and the volume of the parathyroid glands.

Table 1 shows that the relationship between parathyroid size and serum PO_4 level is not a close one over the entire range, due to the low maximal level of serum PO_4 attainable. The apparent dependence of parathyroid volume on serum PO_4 concentration in younger animals was possibly due to the much higher maximum attainable at that age (Carnes et al., '42). Under the present conditions no such dependence is apparent.

It has been suggested, on the basis of considerable circumstantial evidence that the stimulus for the parathyroid glands to produce more hormone is a serum Ca ion level below normal (Albright, '42). The chief factor influencing the ionization of serum Ca is the serum protein concentration (McLean and Hastings, '35). The serum protein was not determined in all the groups of this experiment but in groups 6, 7, and 10 (diets 918, 907, and 909) the average value was 6.6, 6.6, and 6.8%, respectively, and in unpublished experiments on rats of different ages on diets 908 and 913, no significant deviations in serum protein

concentration have been encountered in well-nourished animals. It is unlikely that the serum PO_4 concentration influences the ionization of serum Ca under the present experimental conditions. McLean and Hastings ('35) found no evidence of a non-ionized Ca-PO_4 complex in the plasma under a variety of experimental and spontaneous pathological conditions. Such a complex is producible in the blood under certain conditions, however (McLean and Hinrichs, '38) and the development of manifest tetany in the parathyroidectomized dog has been said to be closely correlated with a critically low ratio of serum Ca/serum PO_4 (Reed et al., '28). It has also been suggested that the parathyroid hyperplasia in renal disease is a result of a disturbance in the Ca/P ratio of the plasma (Jaffee and Bodansky, '43). The table contains the serum Ca/PO_4 ratios of most of the groups in the present experiment. When the various suboptimal diets (groups 1, 2, 8, 9) are considered, a rather close inverse proportionality is observed between this ratio and the parathyroid volume. But this is no closer relationship than that between the serum Ca and parathyroid size in the same groups and the alignment of these groups with those on the more nearly optimal diets (groups 3, 4, 5) is poor. Moreover, the difference in serum Ca/PO_4 ratio between some of the animals with greatly enlarged parathyroids and those with normal-sized glands is not great enough to be significant.

Whereas the present experiment does not yield a decisive answer to the fundamental question of what constitutes the physiological stimulus to parathyroid activity, the data do indicate that over a wide range, both above and below the normal, there is a close correlation between the total serum Ca concentration and parathyroid volume under closely controlled conditions. The possibility remains that the Ca ion concentration is the important moiety. These results are essentially in accord with those of Ham and associates ('40) who concluded that hypocalcemia is the stimulus to physiological hypertrophy of the parathyroid glands. It may be anticipated that other factors, especially the specific growth essentials, will influence the translation of this stimulus into anatomical enlargement. The failure to obtain high enough values of serum PO_4 in this experiment prevents any further conclusions on the possible influence of serum PO_4 level on the size of the glands under other circumstances.

CONCLUSIONS

A close direct proportionality has been found between the logarithm of the dietary Ca/P ratio and the serum Ca concentration in mature rats

on an adequate basal ration over a very wide range of dietary Ca and P. A similarly close inverse proportionality has been found between the logarithm of the dietary Ca/P ratio and the volume of the parathyroid glands. The parathyroid volume and serum Ca concentration accordingly varied inversely and with a nearly linear relationship between serum Ca levels of 7.3 and 11.9 mg. %. The serum inorganic PO_4 level was less uniformly related to the parathyroid size under the present experimental conditions.

LITERATURE CITED

ALBRIGHT, F. 1942 Glandular Physiology and Therapy. Chicago, p. 443.

BAUMANN, E. J., AND D. B. SPRINSON 1939 Hyperparathyroidism produced by diet. Am. J. Physiol., vol. 125, p. 741.

BETHKE, R. M., C. H. KICK AND W. WILDER 1932 The effect of the calciumphosphorus relationship on growth, calcification and blood composition of the rat. J. Biol. Chem., vol. 98, p. 389.

CARNES, W. H., A. M. PAPPENHEIMER AND H. C. STOERK 1942 Volume of parathyroid glands in relation to dietary calcium and phosphorus. Proc. Soc. Exp. Biol. Med., vol. 51, p. 514.

CLARK, E. P., AND J. B. COLLIP 1925 A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., vol. 63, p. 461.

HAM, A. W., N. LITTNER, T. G. H. DRAKE, E. C. ROBERTSON AND F. F TISDALL 1940 Physiological hypertrophy of the parathyroids, its cause and its relation to rickets. Am. J. Path., vol. 16, p. 277.

JAFFE, H. L., AND A. BODANSKY 1943 Serum calcium: clinical and biochemical considerations. J. Mt. Sinai Hosp., vol. 9, p. 901.

KRAMER, B., AND J. HOWLAND 1932 Factors which determine the concentration of Ca and of inorganic P in the blood serum of rats. J. Nutrition, vol. 5, p. 39.

KUTTNER, T., AND HARRIET R. COHEN 1927 Micro colorimetric studies. I. A molybdic acid, stannous chloride reagent. The micro estimation of phosphate and calcium in pus, plasma, and spinal fluid. J. Biol. Chem., vol. 75, p. 517.

MCLEAN, F. C., AND A. B. HASTINGS 1935 The state of calcium in the fluids of the body. I. The conditions affecting the ionization of calcium. J. Biol. Chem., vol. 108, p. 285.

MCLEAN, F. C., AND M. A. HINRICHS 1938 The information and behavior of colloidal calcium phosphate in the blood. Am. J. Physiol., vol. 121, p. 580.

PATT, H. M., AND A. B. LUCKHARDT 1942 Relationship of a low blood calcium to parathyroid secretion. Endocrinology, vol. 31, p. 384.

REED, C. I., R. W. LACKEY AND J. I. PAYTE 1928 Observations on parathyroidectomized dogs, with particular attention to the regional incidence of tetany and to the blood mineral changes in this condition. Am. J. Physiol., vol. 84, p. 176.

SHOHL, A. T., AND S. B. WOLBACH 1936 Rickets in rats. J. Nutrition, vol. 11, p. 275.

ZUCKER, L., AND T. F. ZUCKER 1942 A simple time weight relation observed in well nourished rats. J. Gen. Physiol., vol. 25, p. 445.

ZUCKER, T. F., AND B. N. BERG 1943 Calcium deficiency and gastric lesions in the rat. Proc. Soc. Exp. Biol. Med., vol. 53, p. 34.

SOME FACTORS INFLUENCING THE FECAL ELIMINATION OF THIAMINE BY HUMAN SUBJECTS

ANNE WILLIAMSON[1] AND HELEN T. PARSONS
Department of Home Economics, University of Wisconsin, Madison

TWO FIGURES

(Received for publication July 20, 1944)

Studies of thiamine metabolism in human subjects have, for the most part, been focused upon data obtained from measurement of the urinary excretion, plasma level, or tissue concentration of the vitamin. Where determinations of fecal thiamine have been made, it has often been difficult to interpret the results in the light of the conditions of the experiment, and little significance has been attached to any relationship between the fecal thiamine output and the state of thiamine nutrition of the individual.

Although the regular incidence of thiamine deficiency symptoms in the absence of dietary sources of thiamine has been reported, recent evidence presented by Najjar and Holt ('43) shows that, in their experience, incidence of deficiency symptoms on a diet low in thiamine was not universal. Subjects who did not show clinical signs of deficiency following a prolonged period of the extremely low thiamine intake furnished by a purified diet appeared to eliminate a larger proportion of "free" thiamine in the feces, as contrasted to the "bound" thiamine released by enzymatic digestion, than did the subjects in whom the deficiency could be recognized. The possibility that thiamine from the body was being excreted into the intestinal tract was explored through the injection of massive doses of the vitamin and ruled out when no increase in the fecal thiamine output resulted. These workers believed that the subjects free from clinical deficiency symptoms were able to utilize the synthesized thiamine and that the diet might well be a determining factor in establishing thiamine requirements.

[1] Wisconsin Alumni Research Foundation Assistant 1943–44. This study was made possible by a grant from the Pineapple Research Institute of Hawaii. The experimental data were for the degree taken from a thesis submitted by Miss Williamson in partial fulfillment of the requirements of Master of Science in Home Economics. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

The effect of the diet upon the synthesis of nutritional factors in the intestinal tract has been recognized since the first demonstration of the phenomenon of refection by Fredericia in 1926. Certain raw starches and dextrin have been found to be most effective in stimulating thiamine synthesis but there is no conclusive evidence known to the authors to show that the rat is able to use this synthesized vitamin if coprophagy is prevented. It has more recently been shown that certain factors resulting from microbiological activity in the tract such as vitamin K and biotin are available to the body. It is possible that thiamine, too, under certain conditions, may be absorbed from the lower portions of the digestive tract.

It is evident that clarification of the role of intestinal synthesis in man requires further study of the diet as a factor in stimulating the synthesis of available thiamine. The fecal thiamine elimination of several groups of subjects on a modified milk diet has been studied in this laboratory; results will be discussed and compared with the observations of other workers.

EXPERIMENTAL

It should be stated that the series of experimental diets, from which these results were obtained, was planned with the primary aim of studying various factors influencing gastro-intestinal motility in connection with another study (Stettler, '44). They are equally valid, however, in providing data for a consideration of some of the factors which appeared to influence the thiamine content of the feces.

The low fiber basal diet used was composed of milk, butter, eggs, ice cream, Cheddar cheese and unenriched white bread and supplies about 0.6 mg. of thiamine.[2]

Subjects were graduate and senior women in Home Economics at the University of Wisconsin; that they were satisfactory subjects for this study was indicated by the uniformity of response of urinary thiamine excretions during the first days of the period of controlled level of thiamine intake.

Group 1 consisted of twelve subjects who were given supplements of 600 gm. of drained crushed pineapple or 600 ml. of pineapple juice in addition to the basal diet in order to determine the effect of fiber content of the diet upon thiamine absorption and fecal thiamine elimination. For six of the subjects, the 7-day fiber period preceded the juice period of the same duration; the reverse sequence was followed by the other

[2] Composition: milk — 810, egg — 50, ice cream — 100, Cheddar cheese — 50, bread (white, unenriched) — 125, and butter — 30 gm.

six. Inasmuch as the pineapple juice was obtained by draining the crushed pineapple, the content of soluble constituents was presumably similar (both juice and fiber were found to contain the same concentration of the thiamine); hence, the fiber in the diet was the principal difference between the two periods. Basal diet and pineapple supplements supplied a thiamine intake of 1.3 mg./da.

In the studies with subjects of group 2, an entirely different type of diet, based on meat, was compared to this same modified milk basal in two 5-day periods. The milk, eggs, and cheese of the basal diet of the first period were replaced, during the second, by 300 gm. of ground cured ham [3]; whole crushed pineapple, bread, butter and ice cream were included in both. Although the ham diet supplied appreciably more thiamine, 3.2 as contrasted with 1.3 mg./da., certain comparisons could be made.

Subjects of group 3 were given thiamine chloride supplements of 2 to 10 mg. for 30- to 60-day intervals between two test periods during which the basal diet with supplements of pineapple juice supplying 0.3 mg. thiamine was given so that the total thiamine intake was about 1.0 mg.

Fecal collections were separated by means of carmine markers and daily 24-hour urine collections were made. Ten per cent food aliquots were assayed periodically for thiamine. All determinations were made by means of the thiochrome method, essentially that of Hennessey ('42).

RESULTS

That the substitution of crushed pineapple for pineapple juice in the diet had a marked effect upon the thiamine content of the feces may be seen from table 1. In nine of the twelve subjects, irrespective of sequence of periods, the fecal thiamine was significantly increased above that on the juice period when the coarse pineapple fiber was fed (table 1). Although this is in agreement with the observation of Knott and Schlutz ('39) that the low fiber milk diet produced a lower fecal thiamine content than did the diet to which cereal roughage was added, the lack of variation in the urinary thiamine excretion does not tend to support her hypothesis.

The urinary thiamine excretions of the subjects did not vary significantly with respect to individual subject, to fiber content of the diet, or to sequence of periods as may be seen from table 1. Inasmuch as more than one-half of the total thiamine intake was supplied by the

[3] Supplied through the courtesy of Professor Elvehjem from experimental material in his laboratory.

crushed pineapple and juice supplements to the basal, it would seem that, if interference of the fiber residues with absorption were present, it would be reflected in the level of urinary thiamine excretion. Despite the fact that much of the stool voided during the ingestion of the fiber appeared to contain large quantities of apparently intact pineapple tissue, no consistent trend toward lowered urinary thiamine excretion could be traced. There was no apparent correlation in most instances between the urinary and fecal thiamine eliminations (table 1); even in the case of subjects D. R., where the fecal thiamine output was as low as

TABLE 1

Urinary and fecal thiamine eliminations of twelve subjects of group 1 on a basal diet with low and high fiber supplements.

SUBJECT	THIAMINE				SUBJECT	THIAMINE			
	Urinary when diet contained pineapple		Fecal when diet contained pineapple			Urinary when diet contained pineapple		Fecal when diet contained pineapple	
	drained crushed	juice	drained crushed	juice		drained crushed	juice	drained crushed	juice
	μg./day		μg./day			μg./day		μg./day	
When fiber period preceded the juice period					When juice period preceded the fiber period				
DR	204	208	9	9	MS	300	259	351	186
KO	195	203	902	337	CL	234	263	476	309
RB	263	230	484	370	GJ	299	210	615	252
EB	196	299	341	241	EL	250	316	332	298
SL	285	300	40	74	CM	247	260	246	339
HW	200	240	514	92	EO	231	244	336	221
Av.	224	247	381	187	Av.	260	259	393	267
					Grand average	242	253	387	227

8 μg./da., and K. O., who reached a 902 μg. level during the fiber period, urinary thiamine levels were remarkably similar, 206 and 200 μg./da, respectively. Sequence of periods appeared to have no definite influence thus giving validity to the use of short periods such as these, often necessary in human dietary studies.

It was thought that various data collected during the course of laxation studies carried out simultaneously might be of significance, inasmuch as the character and amount of fecal material is believed to be related to the kind and activity of the intestinal flora. On the basis of dry weight of feces, the residues of bacteria account for a large proportion of the fecal mass; likewise the fate of a complex carbohydrate ingested,

such as cellulose, may, in part, be determined by the intestinal flora. Data were examined to see whether correlations existed between the daily fecal thiamine elimination and such factors as the bulk of feces on the basis of dry weight, moisture content, time for passage of carmine marker and possible individual variations between subjects.

Figure 1 illustrates the relationship between the total thiamine content of the feces per day, dry weight of the fecal material, and the concentration of thiamine per gram of dried feces for both fiber and juice periods. From this series arranged in ascending order of fecal thiamine, it may be seen that the trend of larger fecal outputs parallels, in gen-

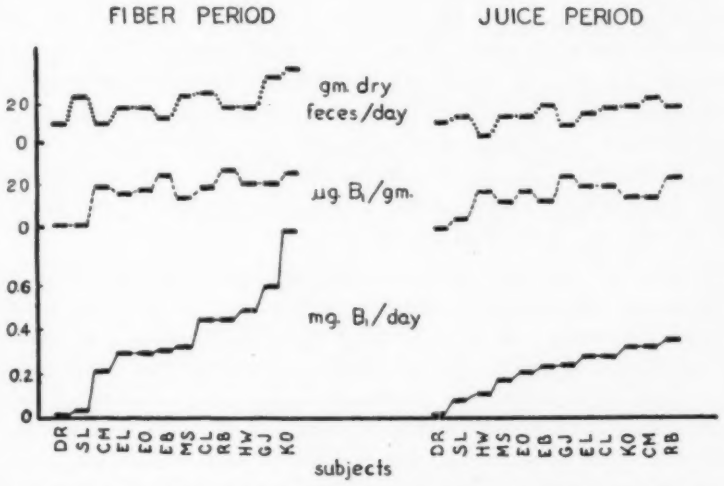


Fig. 1 Relationships between fecal thiamine elimination, dry weight of feces, and concentration of fecal thiamine on fiber and juice supplemented periods.

eral, the increase in fecal weight, the concentration of thiamine remaining relatively constant. Unusually high thiamine output appeared to be associated with greater bulk of feces. This was found to be true for rats by Leong ('37) and, inasmuch as the bulk of the feces is considered to be determined largely by numbers of bacteria, the thiamine content of the feces may be inferred to be related to numbers and activity of organisms in the tract synthesizing thiamine.

It has been shown (Stettler, '44) that the tendency of the crushed pineapple additions to the diet to increase the bulk of the feces on the basis of dry weight was far greater than could be accounted for by the actual amount of fiber ingested during this period as contrasted to the

juice period. Thus the pulp appeared to stimulate bacterial activity and, simultaneously, the fecal thiamine content.

That the additional thiamine found under these circumstances was a result of bacterial activity, rather than incomplete absorption higher in the tract, was further indicated by calculations based on the possible thiamine content of an amount of pineapple equal to the difference in weight of feces as voided between crushed pineapple and juice periods. The added thiamine found in the feces on the fiber period could not be accounted for satisfactorily by the amount of thiamine that would be contained in pineapple passing through the tract.

Walker and Nelson ('33) have shown that, of a number of plant cells tested, intact yeast cells alone retained significant amounts of thiamine not available for absorption when fed fresh without heating.

The concentration of fecal thiamine varied from 13 to 26 μg./gm. in most instances (fig. 1) and is unusual only in two subjects, D. R. and S. L., for whom the range was 0.8 to 5.7 μg./gm. It may be seen that the amount of feces on the dry weight basis was not exceptionally low in either case (fig. 1) so that the extremely low total thiamine output resulted from the low concentration.

Of a total of some twenty-five subjects for whom determinations of fecal thiamine elimination have been made in this series in connection with various studies, five have been characterized by extremely low levels of thiamine in the feces. Even during periods of ingestion of certain types of fresh yeast, ordinarily tending to elevate greatly the fecal thiamine content, two subjects maintained consistently low levels. Three of the five have taken part in several diet studies and in all test periods retained this same uniqueness of response. In these individuals, some special condition apparently existed in the digestive tract which resulted in this particular effect, possibly the destruction of thiamine by organisms or other influences prevailing in the tract itself.

It might be thought that the slower passage of the intestinal contents with an accompanying increase in water absorption would lower the moist fecal weight and possibly the thiamine content of the feces. Comparison between lengths of time for carmine passage and relative fecal moisture and thiamine content indicated, however, that no consistent relationship between these factors occurred in the twelve subjects. It should be noted in this connection, however, that fluoroscopic studies have shown that the time for passage of a carmine marker is not necessarily indicative of the speed of passage of material through any particular portion of the tract.

In general, it is concluded that the influence of the crushed pineapple in elevating the thiamine content of the feces is through the stimulation of intestinal synthesis. Large amounts of water, sugars, and other water soluble constituents held in the tract, in addition to the cellulose, would, presumably, create a favorable medium and would tend to encourage growth of microorganisms.

In the above experiments, a relatively limited diet with additions of known factors such as pineapple fiber has been used. It is of interest to compare the results with those obtained from an entirely different type of diet. After a preliminary 5-day period on the pineapple supplemented milk basal, subjects of group 2 were given the modified milk diet with equal amounts of crushed pineapple and juice. Immediately thereafter, 300 gm. of ground cured ham was substituted for the milk, eggs and cheese of this diet for another 5-day period.

TABLE 2

Average daily urinary and fecal thiamine outputs of four subjects of group 2 on milk and meat basal diets.

	URINARY THIAMINE		FECAL THIAMINE	
	Milk basal [1]	Meat basal [2]	Milk basal [1]	Meat basal [2]
Amount	272 μg.	759 μg.	341 μg.	312 μg.
Percentage of intake	19%	24%	24%	10%

[1] 1.4 mg. intake.
[2] 3.2 mg. intake.

Despite the higher thiamine intake during the meat period (3.2 as contrasted with 1.4 mg. on the milk period) the absolute amount of thiamine in the feces was not increased above that found during the preceding period. It has been commonly thought that meat influences markedly the character of the intestinal flora; if this was the case in this instance, it does not appear that this change affected the fecal thiamine content (table 2). Any influence of the unusually high salt content (10 gm.) of the diet could not be evaluated. The percentage of the thiamine intake excreted in the urine at this level of intake was within the expected range.

Although in nine of the twelve subjects of group 1 variations in fecal thiamine output between fiber and juice periods were inversely related to the urinary thiamine excretions, three subjects did show a striking relationship between urinary and fecal thiamine outputs in response to

the crushed pineapple additions to the diet (fig. 2). Subjects G. J., M. S. and R. B. exhibited higher urinary thiamine excretions during the fiber period; the increments in fecal and urinary thiamine for this period over those of the juice period were roughly proportional. However, amounts of total fecal thiamine for these subjects were no greater than for subjects K. O. and H. W. who had excreted lower amounts of urinary thiamine on the crushed pineapple period despite the high fecal output. Following the procedure of Najjar and Holt ('43), determinations of free thiamine were made in the feces of subjects G. J. and K. O., whose

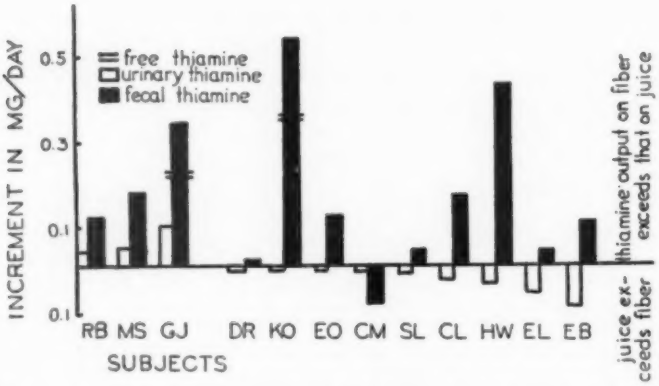


Fig. 2 Degree to which a supplement of crushed pineapple or pineapple juice is associated with an excess of urinary or fecal thiamine. It is to be noted that there is little apparent correlation between urinary and fecal thiamine in this respect.

fecal thiamine outputs had been markedly increased by the inclusion of pineapple fiber in the diet but who differed in response of urinary thiamine excretion. In both subjects, it was found that the free thiamine constituted about 50% of the total and that the difference between fiber and juice periods was no greater for G. J. than for K. O. This leaves questionable the relation of this work to the interpretation of Najjar and Holt that some subjects were able to utilize thiamine synthesized in the digestive tract.

SUMMARY

1. The ingestion of large amounts of plant fiber (pineapple) tended to be associated with relatively large fecal thiamine eliminations. Interference with absorption was not indicated so much as stimulation of intestinal synthesis of thiamine.

2. In general, no correlation was found between variations in urinary and fecal thiamine output for high and low fiber periods, even when there existed in the fecal output a difference great enough so that variations in urinary excretion might be reflected.

3. There were indications that in certain subjects there was destruction of considerable amounts of thiamine in the digestive tract.

LITERATURE CITED

HENNESSEY, D. J. 1942 A standard thiochrome assay for the determination of thiamine in cereal products. Cereal Chem. Bull., vol. 2, no. 2.

KNOTT, E. M., AND F. W. SCHLUTZ 1939 Factors affecting the excretion and requirement of vitamin B_1 in small children. Proc. Am. Inst. of Nutrition, J. Nutrition, vol. 17, p. 12.

LEONG, P. C. 1937 A quantitative study of the metabolism of vitamin B_1 in rats. Biochem. J., vol. 31, p. 373.

NAJJAR, V. A., AND L. E. HOLT 1943 The biosynthesis of thiamine in man. J. Am. Med. Assn., vol. 123, p. 683

STETTLER, H. 1944 The laxative value for human subjects of pineapple juice and pineapple fiber in a low residue basal diet. M. S. Thesis, University of Wisconsin, Madison.

WALKER, R., AND E. M. NELSON 1933 Fresh and dried yeast as sources of vitamin B_1. Am. J. Physiol., vol. 103, p. 25.

STUDIES ON THE CAROTENOID AND VITAMIN A LEVELS IN CATTLE

I. SEASONAL CHANGES OF THE CAROTENOID AND VITAMIN A LEVELS AND THE NORMAL CAROTENOID-VITAMIN A RATIO OF THE BLOOD

WERNER BRAUN[1]

Division of Veterinary Science, University of California, Berkeley

TWO FIGURES

(Received for publication May 1, 1944)

INTRODUCTION

Various investigators have reported on the carotenoid and vitamin A content of the liver and blood of men and animals under different dietary and pathological conditions (Barron, '42; Boyer et al., '42; Braun and Carle, '43; Davis and Madsen, '41; Deuel et al., '42; Gallup and Kuhlman, '41; Guilbert and Hart, '34, '35; Keener et al., '42; Lindquist, '38; Ralli et al., '41; With, '40).[2] A few of these investigators, especially Stepp and Wendt ('37), have made an effort to correlate the carotenoid and vitamin A levels in human blood and were unable to obtain consistent results. This failure is probably due to the uncertain intake of vitamin A itself, which masks the results of such studies on human material. In cattle, if maintained without vitamin A supplement, this complicating factor is eliminated, since all the vitamin A present in the blood has been converted from carotenoids within the animal. Studies on cattle may thus contribute to a better knowledge of the normal ratio of carotenoids to vitamin A in the blood as well as in the liver.

Such information, as well as other data on vitamin A metabolism, have been obtained in an experiment which had been primarily designed to test the effect of dietary regimes upon the resistance to Brucella abortus infection in cattle.[3] During this experiment, nineteen different blood constituents of forty-nine cows maintained in four dietary

[1] Agent (Cooperative), United States Bureau of Animal Industry at the Division of Veterinary Science, University of California, Berkeley, California.

These studies were supported in part by grant from the U. S. Bureau of Animal Industry under cooperative agreement with the Regents of the University of California.

[2] For further references, see chapter on fat-soluble vitamins in "Annual Reviews of Biochemistry," published since 1932 by Annual Reviews, Inc., Stanford Univ.

[3] This main project has been conducted by Dr. C. M. Haring, Dr. J. Traum and the author.

groups with different carotenoid and vitamin A intake, were repeatedly tested for a period of 1 year, among them tests for carotenoid and vitamin A. This report will present a brief discussion of the seasonal changes observed for the carotenoid and vitamin A levels in the blood of these animals and an analysis of their carotenoid-vitamin A ratios.

EXPERIMENTAL ANIMALS

All animals were maintained on nonirrigated, natural grass pasture in Strawberry Canyon, Berkeley, California, without supplementary feed from March until September. After September 1, the animals were divided into four groups: (1) the pasture group (P) consisted of animals which remained on the same pasture without supplementary feed throughout the year; (2) a pasture group (PA) of animals which were maintained on the same pasture as the P group but were fed 400,000 I.U. of vitamin A in the form of shark-liver oil twice a week from September through November; (3) a group of animals which were transferred from pasture into the barn (B group) and was maintained there on a carotenoid and A-deficient diet consisting of beetpulp, cottonseed-meal and rolled barley; (4) a group of barn animals which received a shark-liver oil supplement (400,000 I.U. twice a week) from September through November (BA groups).

The animals varied in age from 1 to 13 years. The majority were Holstein cows, but there were also a few Jerseys, 1 Hereford, and a few cross-bred animals.

On October 2nd, the pregnant animals were exposed to Brucella abortus. Most of the nonvaccinated animals and a number of the vaccinated animals aborted during November and December. The infection with Brucella abortus had no effect on the blood constituents tested including the carotenoid and vitamin A levels, except those changes which were produced during subsequent abortions; therefore, the results obtained can be used for a general analysis of carotenoid and vitamin A changes.

METHODS

The carotenoid and vitamin A content of the blood was measured with the Cenco photometer, using the method described by Kimble ('39). Wratten filter no. 47 was used for the reading of carotenoids and Wratten filter no. 58 for the blue color developed after addition of $SbCl_3$ in chloroform to the prepared sample. Kimble's calculation for vitamin A and carotenoid corrections was used. A preparation of pure β-carotene was used for the standardization of the carotenoid readings. It

is realized that the data for vitamin A, as expressed in I.U./ml. or I.U./gm. are probably only relative, but this does not affect the results of these comparative studies.

RESULTS AND DISCUSSION

Seasonal changes of the carotenoid and vitamin A levels

From March to September 1st all animals were on pasture and showed decreasing carotenoid levels in successive tests made during this period. A tendency for carotenoid levels of old animals (older than 1 year and 6 months) to remain at high levels longer than those of young animals (younger than 1 year and 6 months) was noted but this difference did not reach statistical significance in an analysis of variance (Snedecor, '34) based upon all data for carotenoid levels between March and September.[4] Differences between means of tests, i.e., seasonal changes, however, were found to be highly significant. A significant difference was also revealed to exist between the means of animals, i.e., individual differences, partly due to differences in breed of animals, which caused differences in maximum levels (= highest level found during 1 year of testing) and time at which the decrease of carotenoid levels started.

The rapid decrease of carotenoid levels lasted until August. Animals which remained on pasture after September 1st (P group) maintained low levels until November and showed a rapid increase in carotenoid levels after this date. Those animals which were maintained on the same pasture but received a vitamin A supplement (PA group) showed on the average lower carotenoid levels than the animals of the P group. This confirms observations of Deuel, Hallman and Mattson ('42) that feeding of vitamin A lowers the carotenoid level.

Animals of the B group continued to show a decrease of their blood carotenoid level until November, when no carotenoids could be detected in the blood by methods used here. Administration of vitamin A (BA group) showed no effect on these low levels.

As in the case of carotenoids, vitamin A levels dropped in pasture animals from about March until August, remained low until November, and increased after that date. An analysis of variance based on March-September data revealed no significant difference between age groups, highly significant differences between means of tests, i.e., seasonal changes, and no significant differences between means of animals (in-

[4] The advice of Dr. I. M. Lerner, Division of Poultry Husbandry, regarding the statistical treatment of these data is gratefully acknowledged.

dividual differences), although tendencies for differences between animals of different breed were indicated.

Vitamin A levels increased immediately after administration of shark-liver oil to pasture animals (PA group). Animals of the B group continued to show decreasing vitamin A levels until values near zero were found 4 to 5 months after feeding of the carotenoid and vitamin A-deficient diet was begun. Animals receiving shark-liver oil supplements in addition to this diet (BA group) maintained higher blood levels of vitamin A, which dropped to levels observed in B animals immediately after cessation of vitamin A supplements.

Vitamin A levels of the blood were not only dependent on vitamin A intake and carotenoid levels but also on changes in certain physiological factors, like infection, abortion and calving. Such factors produce fluctuations of the vitamin A level of the blood which are independent from the fluctuations of the carotenoid level. Thus, a significant and sharp drop of vitamin A levels started approximately 2 weeks before parturition or abortion, reached its lowest level (minimum level) a few days after parturition or abortion, when it was followed by a sudden rise to the former level. In many animals no vitamin A at all was found in the blood at this minimum shortly after parturition or abortion,[5] particularly in animals of the B and BA groups, less so in animals of the P and PA groups, where the average minimum level reached a point slightly above the zero level.[6] Certain pathological conditions affected the vitamin A level similarly. These pathological conditions were mainly acute infections, like localized abscesses or gangrenous mastitis. In these cases a sharp drop of vitamin A levels was observed, usually before the symptoms manifested themselves, and normal levels were only restored when the infection subsided. The administration of vitamin A in large doses did not prevent these sudden decreases observed in aborting and calving animals of the PA and BA groups, thus ruling out disturbances of vitamin A conversion as the sole underlying cause for the low blood levels.

Figure 1 attempts to present in a composite graph the foregoing discussed changes of vitamin A and carotenoid levels of the blood.

[5] Zero values for vitamin A may represent a failure in technique rather than complete absence of vitamin A.

[6] An extended investigation of this change at time of calving has been made on lactating cows in order to ascertain whether this change is of general occurrence. It was found that the same drop of the vitamin A level around time of calving took place in lactating animals, too. The results of this additional study will be published elsewhere.

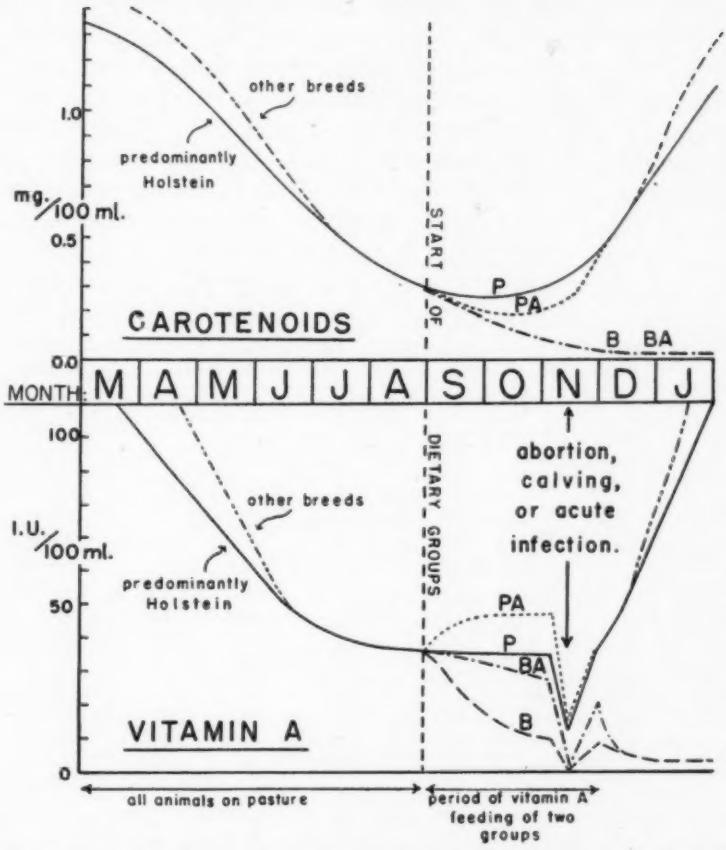


Fig. 1 Diagram showing average changes of carotenoid and vitamin A levels of the bovine blood with changing diet, and during abortion, calving or acute infections. For explanation of dietary groups see text.

The relation of carotenoids to vitamin A in the blood

Since the experiment here reported yielded data on various carotenoid levels with their corresponding vitamin A levels it was possible to analyze the relationship between the two levels under known conditions of carotenoid and vitamin A intake as well as at times of physiological disturbances. Figure 2a illustrates this relationship as found in data obtained from animals which did not receive a vitamin A supplement; therefore, the only source of vitamin A in their blood was the carotenoid intake which had been converted into vitamin A in the liver.

The abcissa of figure 2a represents the carotenoid values; the ordinate, the corresponding vitamin A values. Each point represents the carotenoid and vitamin A value for one test. (Only data obtained from tests made during December to April, when the carotenoid levels generally increased, are used in the graph for reasons to be discussed below.) The distribution of points indicates a linear increase of vitamin A with increasing carotenoid levels (a result, which is not supported by previous work reported by others on cattle). From this graph, a table was constructed which shows the average vitamin A level for each carotenoid level (table 1). By dividing each vitamin A value by its corresponding carotenoid value, a ratio was obtained which indicates in one figure the relative relationship, but should not be interpreted as

TABLE 1

Minimum normal $\frac{A}{car}$ *ratios.*

CAR MG./100 ML.	A I.U./100 ML.	$\frac{A}{CAR}$ RATIO	CAR MG./100 ML.	A I.U./100 ML.	$\frac{A}{CAR}$ RATIO	CAR MG./100 ML.	A I.U./100 ML.	$\frac{A}{CAR}$ RATIO
0.05	25	500	0.50	51	102	0.95	87	92
0.10	26	260	0.55	55	100	1.00	91	91
0.15	27	180	0.60	59	98	1.05	95	90
0.20	29	145	0.65	63	97	1.10	100	90
0.25	31	124	0.70	67	96	1.15	104	90
0.30	35	117	0.75	71	95	1.20	108	90
0.35	39	111	0.80	75	94	1.25	112	90
0.40	43	108	0.85	79	93	1.30	116	89
0.45	47	104	0.90	83	92	1.35	120	89

a direct quantitative measurement between corresponding levels because vitamin A is expressed in I.U./100 ml. and carotenoids in mg./100 ml. here. This will be called the "$\frac{A}{car}$ ratio." Table 1 shows that the $\frac{A}{car}$ ratio decreases with increasing carotenoid levels, reaching a rather constant value around 90 above carotenoid levels of 0.9 mg./100 ml. This decrease of the $\frac{A}{car}$ ratio is graphically indicated in figure 2b. The decrease in the ratio with increasing carotenoid levels may be due to decreasing efficiency of conversion of carotenoids into vitamin A with increasing intake and/or a decreasing release of vitamin A stores from the liver with increasing carotenoid levels.

Deviations from the typical $\frac{A}{car}$ ratios were found under certain conditions. In animals which did not receive vitamin A as such, $\frac{A}{car}$ ratios lower than normal were found during abortions and parturitions, as well as at time of known pathological disturbances. In figure 2a the crosses

indicate carotenoid and corresponding vitamin A levels at time of abortion or parturition. These points lie well below the normal distribution, since the vitamin A values are lower than would be expected for each carotenoid level. During the discussion of vitamin A changes at times of abortion and parturition, it has been shown that the vitamin A level dropped considerably. The calculation of the $\frac{A}{car}$ ratio now shows that it is unnecessary to follow this drop in vitamin A by a number of tests in order to show the decrease of the vitamin A level of the

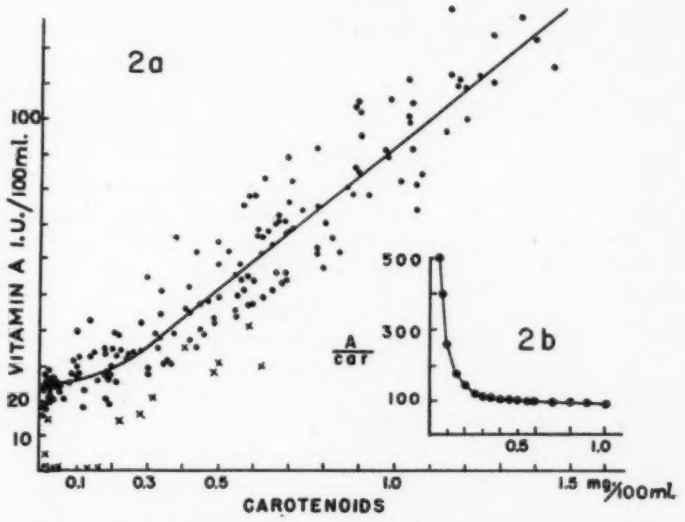


Fig. 2 *a*: Correlation of carotenoids and vitamin A in the blood. Abscissa: carotenoid values; ordinate: corresponding vitamin A values. *b*: Graphical representation of the decrease of the $\frac{A}{car}$ ratio with increasing carotenoid levels. Abscissa: carotenoid values: ordinate: $\frac{A}{car}$ ratios.

blood. One test at the critical time is sufficient to indicate a deviation from the normal $\frac{A}{car}$ ratio and thus establish the presence of physiological disturbances.

$\frac{A}{car}$ ratios above normal were encountered at various times and are best illustrated by a few examples. During a period of steady increase in carotenoids, the $\frac{A}{car}$ ratio follows the normal decrease of values presented in table 2. Upon inspection of a series of values obtained from tests at more frequent intervals, it becomes apparent that the $\frac{A}{car}$ ratio increases above normal for the respective carotenoid level as soon as the carotenoid level drops (see examples in table 2). (This probably means that a decrease of the carotenoid level causes an immediate re-

lease of additional vitamin A stores from the liver.) Apparently it does not matter from which level the carotenoid decrease starts. The $\frac{A}{car}$ ratio always increases at such time, regardless of the size of the carotenoid level. This is the reason why it was mentioned above that only data obtained during periods of increasing carotenoid levels were used in establishing the normal $\frac{A}{car}$ ratio. It should be recognized that the initial rise of the $\frac{A}{car}$ ratio at times of carotenoid decrease is due to the

TABLE 2

Examples of the effect of increasing and decreasing carotenoid levels upon the $\frac{A}{car}$ ratio.

COW NO.	DATE OF TEST	CAR MG./ 100 ML.	A I.U./ 100 ML.	$\frac{A}{CAR}$ RATIO	COW NO.	DATE OF TEST	CAR MG./ 100 ML.	A I.U./ 100 ML.	$\frac{A}{CAR}$ RATIO
Increasing carotenoid levels									
313	12/14/42	0.14	42.5	304	273	12/14/42	0.19	27.7	146
	1/ 4/43	0.50	48.7	97		1/ 5/43	0.28	34.4	123
321	11/10/42	0.28	41.7	149	279	12/ 4/42	0.34	51.5	150
	1/13/43	1.52	145.4	96		3/19/43	0.89	85.8	96
Decreasing carotenoid levels									
4	2/ 9/43	0.90	95.3	106	308	2/19/43	0.97	91.4	94
	2/16/43	0.99	105.4	106		2/26/43	1.04	100.1	96
	3/10/43	0.58	105.9	183		3/ 9/43	0.44	64.0	145
302	1/29/43	0.26	33.7	130	316	1/21/43	1.24	111.8	90
	2/ 3/43	0.24	33.1	138		2/ 5/43	1.18	108.8	92
	2/ 9/43	0.34	43.9	129		2/12/43	1.14	125.4	110
	2/16/43	0.45	54.8	122		2/17/43	1.16	137.9	119
	3/ 8/43	0.22	41.1	187		3/ 9/43	0.42	52.2	124
292	2/ 3/43	0.44	62.1	141	312	2/ 9/43	0.78	80.7	103
	2/18/43	0.63	83.3	132		2/18/43	1.16	111.5	96
	2/26/43	0.78	74.7	96		2/26/43	1.28	123.4	96
	3/ 8/43	0.45	62.6	139		3/10/43	0.70	125.7	180
	4/ 6/43	0.71	75.0	106		4/ 7/43	1.20	110.1	92

fact that the vitamin A level does not exactly correspond to the carotenoid level tested at the same time. In other words, the vitamin A found in the blood originates from carotenoids carried by the blood to the liver some time ago where it has been converted first before appearing as vitamin A in the blood. The vitamin A level, therefore, will show changes which actually correspond to previous carotenoid changes. However, the increase of the $\frac{A}{car}$ ratio at times of decreasing carotenoid levels, as illustrated in table 2, persists for longer periods than could be

attributed to the difference in time of corresponding carotenoid and vitamin A levels and some other mechanism, such as the suggested additional output of liver vitamin A reserves, must be held responsible. This is further substantiated by a subsequent test which revealed that the principle of increased ratios at time of decreasing carotenoid levels apparently does not apply to animals with fairly depleted vitamin A stores. Four of the animals which had been maintained on the carotenoid and vitamin A-free diet for over 8 months were periodically released on pasture and after a few days returned to the deficient diet. Semiweekly tests on these vitamin A depleted animals subject to fluctuating carotenoid intake revealed no increase in the $\frac{A}{car}$ ratio when the carotenoid levels decreased.

Finally, considerably higher $\frac{A}{car}$ ratios than those compiled in table 1 were always found in animals which received a shark-liver oil supplement. Obviously, these higher ratios are caused by the addition of ingested vitamin A to the normally present vitamin in the blood, since it is known that vitamin A taken into the body with the feed will be absorbed by the blood from the intestines, part of it transported into the liver for storage and a part utilized immediately by the animal (With, '40).[7] Aside from fed vitamin A, at least one other nutritional factor appeared to be able to elevate the vitamin A level of the blood, thereby causing a considerably higher $\frac{A}{car}$ ratio than normal, namely feeding of yeast; it caused an immediate 250 to 300% increase of the vitamin A level after daily feeding of 250 gm. to two animals which had been maintained in the B group for 7 months. Abels, Gorham, Pack and Rhoads ('41) were the first to report this effect in man and suggested that it may be caused by a mobilization of vitamin A stores from the liver due to lipotropic substances contained in the yeast.

It is well known that carotenoid and vitamin A levels differ among breeds (Boyer et al., '42), particularly in the milk, and differences in seasonal changes of the blood level caused by the breed of the animal have been mentioned above. Therefore, the question arises whether these breed differences may not affect the $\frac{A}{car}$ ratio. However, the animals studied in this experiment do not represent a genetically homogenous group; still, the relative $\frac{A}{car}$ ratio did not show any differences for animals of known different ancestry. Furthermore, samples obtained from Hereford and Jersey cattle at the slaughterhouse revealed similar $\frac{A}{car}$

[7] This point is of importance for tests in which the vitamin A status of an animal is being tested by blood-tests. Care has to be taken at the time of such a test to see that no vitamin A is being taken in by the animal since such an intake would mask the actual release of stored or converted vitamin A from the liver.

ratios for corresponding carotenoid levels. Thus it seems that breed differences are expressed in different seasonal levels which apparently do not affect the relative ratio of vitamin A to carotenoids.

SUMMARY

The carotenoid and vitamin A levels of the blood of forty-nine cows maintained in four dietary groups with different carotenoid and vitamin A intake, were determined periodically for 1 year.

The seasonal changes of the carotenoid level were mainly dependent on the diet, i.e., carotenoid intake, but varied according to the age and breed of the animal. Individual difference, i.e., differences between the means of animals, were found to be statistically significant. Pathological disturbances showed no direct effect on the carotenoid level.

The seasonal changes of the vitamin A level were dependent on carotenoid and vitamin A intake and were modified during parturition, abortion and acute infections at which time vitamin A levels showed a sharp, temporary decrease. No statistically significant difference was found between individual animals or age groups, but a difference between breeds was indicated. The vitamin A level rose immediately after administration of shark-liver oil and, according to a preliminary test, after feeding of yeast.

The relation of carotenoids to vitamin A in the blood was analyzed. A linear increase of vitamin A with increasing carotenoid levels was found. The ratio of vitamin A to carotenoids at various carotenoid levels was established. This $\frac{A}{car}$ ratio was found to decrease with increasing carotenoid levels, reaching a constant value at high carotenoid levels. Deviations from the normal $\frac{A}{car}$ ratios were found and analyzed. The $\frac{A}{car}$ ratio was below normal in cases of physiological disturbances (parturition, abortion, acute infections), slightly above normal if animals were subject to decreasing carotenoid intake, and well above normal if a vitamin A supplement was fed. The effects of breed differences on the $\frac{A}{car}$ ratio are briefly discussed.

LITERATURE CITED

ABELS, J. C., A. T. GORHAM, G. T. PACK AND C. P. RHOADS 1941 Metabolic studies in patients with cancer of the gastro-intestinal tract. I. Plasma vitamin A levels in patients with malignant neoplastic diseases, particularly of the gastro-intestinal tract. J. Clin. Med., vol. 20, p. 749.

BARRON, N. S. 1942 Vitamin A and its relationship to some of the commoner diseases of farm animals. Vet. Rec., vol. 54, p. 29.

BOYER, P. D., P. H. PHILLIPS, N. S. LINDQUIST, C. W. JENSEN AND I. W. RUPEL 1942 Vitamin A and carotene requirements for the maintenance of adequate blood plasma vitamin A in the dairy calf. J. Dairy Sci., vol. 25, p. 433.

BRAUN, W., AND B. N. CABLE 1943 The effect of diet on the vitamin A content of the bovine fetal liver. J. Nutrition, vol. 26, p. 549.

DAVIS, R. E., AND L. L. MADSEN 1941 Carotene and vitamin A in cattle blood plasma with observations on reproductive performance at restricted levels of carotene intake. J. Nutrition, vol. 21, p. 135.

DEUEL, H. J. JR., L. F. HALLMAN AND F. MATTSON 1942 The effect of high vitamin A intake on the blood and milk carotene of Holstein and Guernsey cows. J. Nutrition, vol. 23, p. 567.

GALLUP, W. D., AND A. H. KUHLMAN 1941 Carotene content of the blood plasma of dairy cattle in relation to vitamin A deficiency. Okla. Acad. Sci. Proc., vol. 21, p. 89.

GUILBERT, H. R., AND G. H. HART 1934 Storage of vitamin A in cattle. J. Nutrition, vol. 8, p. 25.

———— 1935 Minimum vitamin A requirements with particular reference to cattle. J. Nutrition, vol. 10, p. 409.

KEENER, H. A., S. I. BECHDEL, N. B. GUERRANT AND W. T. S. THORP 1942 Carotene in calf nutrition. J. Dairy Sci., vol. 25, p. 571.

KIMBLE, M. S. 1939 The photocolorimetric determination of vitamin A and carotene in human plasma. J. Lab. and Clin. Med., vol. 24, p. 1055.

LINDQUIST, T. 1938 Studien über das vitamin A beim Menschen. 1938 Upsala. Appelbergs Boktryckeriaktiebolag.

RALLI, E. P., E. PAPPER, K. PALEY AND E. BAUMAN 1941 Vitamin A and carotene content of human liver in normal and in diseased subjects. Arch. Int. Med., vol. 68, p. 102.

SNEDECOR, G. W. 1934 Calculation and interpretation of analysis of variance and covariance. Monograph no. 1, Div. Ind. Sci., Iowa State College, p. 1.

STEPP, W., AND H. WENDT 1937 Einige Beobachtungen über das Verhältniss von Carotin zu Vitamin A im menschlichen Blutserum. Deutsches Archiv f. Klin. Med., vol. 180, p. 640.

WITH, T. K. 1940 Absorption, metabolism and storage of vitamin A and carotene, with some remarks on the vitamin A requirements. Published by Einar Munksgaard and Oxford University Press, London, p. 1.

STUDIES ON THE CAROTENOID AND VITAMIN A LEVELS IN CATTLE

II. CAROTENOIDS AND VITAMIN A IN THE LIVER, THEIR RATIO AND THEIR RELATIONSHIP TO BLOOD LEVELS

WERNER BRAUN [1]
Division of Veterinary Science, University of California, Berkeley

TWO FIGURES

(Received for publication May 1, 1944)

INTRODUCTION

Seasonal changes of the carotenoid and vitamin A levels of the blood of a group of cattle maintained on various dietary regimes were reported in a previous paper (Braun, '45). Data were also obtained on the carotenoid and vitamin A content of the livers of animals used in this study. These data will be discussed in this report, together with additional data obtained from slaughterhouse material which was collected in order to establish the relationship between vitamin A levels of the liver and the blood.

The vitamin A and carotenoids in liver tissue were determined by Guilbert and Hart's ('34) method which was modified for use with the Cenco photometer.

Liver samples were obtained at time of slaughter or, in a few cases, by partial hepatectomy. Blood samples were always collected at the same time and their carotenoid and vitamin A content was determined according to methods described in an earlier report (Braun, '45).

The data obtained from the University of California cows gave information about the effect of vitamin A feeding on the liver storage of vitamin A, because the animals were maintained in four dietary groups: (1) a pasture group (P group); (2) a pasture group in which each animal received 400,000 I.U. of vitamin A, in the form of shark-liver oil, twice a week from September through November (PA group); (3) a barn group, transferred on September 1st from pasture into the barn

[1] Agent (Cooperative), United States Bureau of Animal Industry at the Division of Veterinary Science, University of California, Berkeley, California.

These studies were supported in part by a grant from the U. S. Bureau of Animal Industry under cooperative agreement with the Regents of the University of California.

TABLE 1

Carotenoids and vitamin A in the liver and the corresponding blood levels.

DIETARY GROUP (SEPT.–NOV.)	COW NO.	DATE OF TEST	CAR. MG./GM.	A I.U./GM.	CAR. MG./100 ML.	A I.U./100 ML.
A. Data obtained during and shortly after period of shark-liver oil feeding.						
P	263B	11/ 5/42	13.4	244.7	...	...
	3	11/25/42	6.2	195.8	0.32	21.2 [1]
		2/ 1/43	10.4	262.8	0.61	39.5
	315	12/17/42	7.6	256.8	0.44	45.0
	305	1/ 5/43	8.4	279.2	0.42	51.8
	341	1/ 5/43	10.0	256.3	0.50	65.0
	313	1/ 5/43	7.2	253.2	0.50	48.7
	2	12/17/42	4.8	220.6	0.44	47.9
			M = 246.2			
B	319	12/ 2/42	2.4	120.8	0.00	0.0 [2]
	343	12/15/42	1.4	94.0	0.01	10.5 [3]
	200	1/11/43	1.4	96.8	0.03	19.8
	(284	1/19/43	1.6	181.3	0.02	0.0) [4]
			M (without 284) = 103.9			
PA	273	1/ 5/43	8.4	449.4	0.28	34.4
	326	1/ 5/43	10.0	342.9	0.90	101.6
			M = 396.2			
BA	282	12/ 1/42	7.4	335.8	0.01	0.0 [5]
		2/ 1/43	4.0	341.1	0.01	24.6
	293	12/15/42	2.6	265.8	0.05	0.0 [6]
	318	1/ 5/43	5.2	396.5	0.30	34.8
			M = 334.8			
B. Data obtained 6 to 8 months after cessation of shark-liver oil feeding.						
P	309	5/25/43	16.0	409.5	0.59	61.3
	314	7/15/43	8.0	385.3	0.26	51.1
	342	7/15/43	7.0	333.3	...	...
			M = 376.0			
B	312	5/25/43	20.8	339.4	0.78	99.0
	343	5/25/43	6.4	118.7	0.42	63.6 [7]
	247	7/15/43	4.2	297.4	0.23	33.8
	327	7/15/43	6.2	142.4	0.31	36.4
			M = 224.5			
PA	4	5/25/43	20.4	522.9	0.82	87.5
	207	7/15/43	14.8	578.4	0.45	50.4
			M = 550.3			
BA	(292	5/25/43	9.6	293.1	0.55	60.9) [8]
	1369	7/15/43	8.6	464.5	0.17	27.0

[1] Liver sample obtained by partial hepatectomy after abortion on 11/24/42.
[2] Liver sample obtained by partial hepatectomy after abortion on 12/1/42.
[3] Liver sample obtained by partial hepatectomy after abortion on 12/14/42.
[4] Died in corral. Autopsy revealed severe peritonitis and adherent pericardium, also inflamed areas in intestine.
[5] Liver sample obtained by partial hepatectomy after abortion on 11/28/42.
[6] Extensive edema of abdomen and brisket, with a huge slough in the right axillary region at time of slaughter.
[7] Released on pasture on 3/10/44.
[8] Pericarditis, foreign body lesions revealed by autopsy on 5/25/43.

where they were maintained on a carotenoid and A-deficient diet (B group; and (4) a group of barn animals which received a shark-liver oil supplement (400,000 I.U. twice a week) from September through November (BA group). A more detailed description of these animals, their diet and the changes observed in their carotenoid and vitamin A levels of the blood have been presented previously (Braun, '45).

RESULTS AND DISCUSSION

The values obtained from liver samples of these animals are presented in table 1, where they are arranged according to dietary groups. Data collected during November through January, i.e., during and immediately after the period of shark-liver oil feeding, are grouped separately from data obtained from animals slaughtered 6 to 8 months later. The November–January data were then used for a calculation of ratios of vitamin A between the averages of the different dietary groups (table 2). Values obtained after partial hepatectomy are incorporated in table 1.

TABLE 2

The ratio of average vitamin A storage between the four dietary groups.

GROUP	RATIO OF AVERAGE VITAMIN A BETWEEN THE GROUPS
Barn fed: Barn fed plus shark-liver oil	1:3.5
Barn fed: Pasture fed	1:2.4
Barn fed: Pasture fed plus shark-liver oil	1:4.1
Pasture fed: Barn fed plus shark-liver oil	1:1.4
Pasture fed plus shark-liver oil: Barn fed plus shark-liver oil	1:0.9
Pasture fed: Pasture fed plus shark-liver oil	1:1.6

An inspection of these data indicates: (1) considerable differences in liver vitamin A levels between animals of the B group, the P group and both BA and PA groups, with less significant differences between the two latter groups (upper part of table 1); (2) a rather lasting effect of the relatively short period of vitamin A feeding on the liver storage, since the group differences were still found in animals which had been on identical diets for at least 4 months before slaughter which was 6 to 7 months after cessation of shark-liver oil feeding (lower part of table 1); and (3) an optimum level for vitamin A storage, suggested by the ratios in table 2, which show that levels of the BA group were 3.5 times higher than those of the B group, while those of the PA group were only 1.6 times higher than those of the P group, both groups having received equal amounts of shark-liver oil. (If there would not be an opti-

mum level for vitamin A storage, the vitamin A storage of the PA group should be considerably higher after vitamin A feeding than that of the BA group, because the pasture animals without vitamin A supplement had 2.4 times as much vitamin A in their livers as the barn animals without vitamin A supplement.) Next, it should be noted that uniformly low carotenoid levels were observed only in animals without carotenoid or vitamin A intake (B group). Results obtained with cow no. 282 are particularly interesting in this connection. The caudate liver lobe of this animal had been removed on December 1st [2] and its carotenoid and vitamin A content had been estimated at 7.4 μg./gm. and 335.8 I.U./gm., respectively. Two months later this animal was slaughtered after having been maintained on the carotenoid and vitamin A-free diet of the barn group. At the time of slaughter, the carotenoid level of the liver was found to be 4.0 μg./gm., indicating an almost 50% decrease of the carotenoid content. However, the vitamin A content had remained unchanged; the value obtained was 341.1 I.U./gm. More experimental work appears necessary to substantiate the suggestion that a utilization of stored vitamin A first forces available carotenoids to be converted into vitamin A, thus first decreasing the carotenoid level without decreasing the vitamin A level, and only after a very low carotenoid level has been reached will a drain on vitamin A stores become apparent by decreased vitamin A storage. The increase of liver stores when green feed becomes available on pasture after January is illustrated in the results of partial hepatectomies performed on cows no. 3 and no. 343 (table 1).

Similarly, to the $\frac{A}{car}$ ratios observed in the blood (Braun, '45), a typical relationship between carotenoid levels and corresponding vitamin A levels appears to exist in the liver. The ratio of each vitamin A level to its corresponding carotenoid level was calculated, and the resulting ratios were plotted against increasing carotenoid levels (fig. 1). The distribution of the dots in this graph, representing values obtained from animals without vitamin A supplement, indicate a decrease in the $\frac{A}{car}$ ratio of the liver with increasing carotenoid levels. Each carotenoid level has its typical $\frac{A}{car}$ ratio. The above cited decrease of the carotenoid level without changes in the vitamin A level, i.e., the metabolic tendency of the organism to maintain a constant vitamin A store, probably causes the rapid rise in $\frac{A}{car}$ ratios with decreasing carotenoid levels. The $\frac{A}{car}$ ratios of the liver were modified if the animals were fed vitamin A, as was the case in the animals of the barn and pasture groups which had been fed a shark-liver oil supplement. The circles on the graph represent the values obtained for the $\frac{A}{car}$ ratio of these shark-liver oil-

[2] This operation was performed by Dr. B. N. Carle.

fed animals. The $\frac{A}{car}$ ratio of all these animals is constantly larger for each carotenoid level compared with ratios of animals which did not get the supplement. This can be easily explained: the vitamin A fed has been added to the normally present vitamin A stores, thus modifying the $\frac{A}{car}$ ratio. This modification of the normal $\frac{A}{car}$ ratio of the liver under conditions where vitamin A is supplied with the feed explains why investigators like Ralli et al. ('41) were unable to detect constant $\frac{A}{car}$ ratios in their human liver material. The human diet contains varying

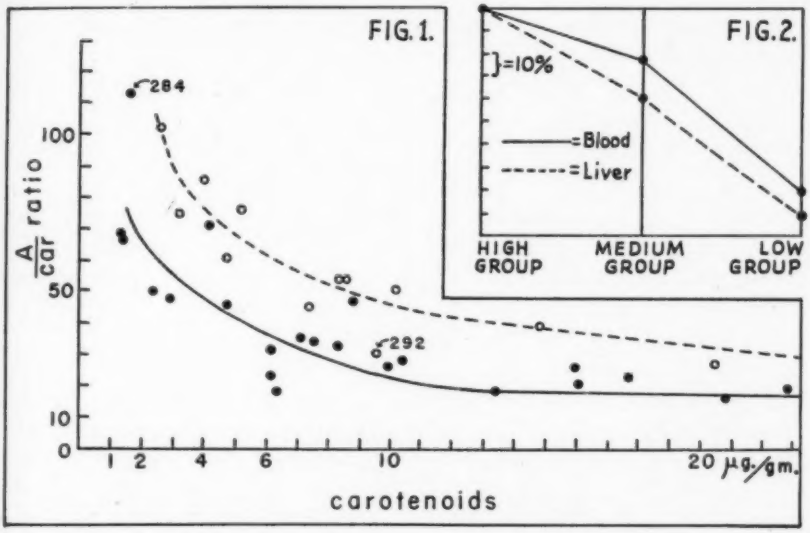


Fig. 1 Graphical representation of the decrease of the $\frac{A}{car}$ ratio of the liver with increasing carotenoid levels. Dots represent data from animals maintained without vitamin A supplement; circles represent data from animals which received vitamin A supplement. Abscissa: carotenoid values; ordinate: $\frac{A}{car}$ ratios.

Fig. 2 Graphical representation of the relationship between vitamin A levels of the liver and those of the blood, using the means of data presented in table 3. Abscissa: dietary groups; ordinate: vitamin A levels in per cent of decrease.

amounts of vitamin A in addition to carotenoids and this fluctuating intake of vitamin A modifies the normal $\frac{A}{car}$ ratio of the liver as well as that of the blood (Braun, '45). The above mentioned observation that temporary feeding of vitamin A had a rather lasting effect on the increased vitamin A storage makes it possible to detect administration of vitamin A supplements, through liver tests and comparison with normal $\frac{A}{car}$ ratios, made an appreciable period after the supplement has been stopped. A number of workers have shown that the liver vitamin

A is decreased under various pathological conditions (Barron, '42; Ralli and co-workers, '41; With, '40). The liver material here investigated contained three specimens obtained from animals at the time of pathological disturbances: namely cows no. 284, no. 292 and no. 293 (table 1). Two of them (no. 284 and no. 292) showed unusual $\frac{A}{car}$ ratios of the liver. Blood samples from cows no. 184 and no. 293 showed an abnormal $\frac{A}{car}$ ratio at the time when the liver samples were obtained. Liver specimens from animals no. 3, no. 282, no. 319 and no. 343 were obtained immediately after abortion. No abnormal $\frac{A}{car}$ ratio was found in these livers at that time, while the $\frac{A}{car}$ ratio of the blood was abnormal at this period (Braun, '45).

TABLE 3

Mean vitamin A levels of the liver and of the blood for three groups of animals maintained on diets with different vitamin A intake.

GROUP	NUMBER OF ANIMALS	MEAN OF VIT. A IN LIVER	MEAN OF VIT. A IN BLOOD
High group	37	69.2 ∓ 5.53	20.4 ∓ 1.18
Medium group	6	42.8 ∓ 4.09	16.1 ∓ 1.65
Low group	9	19.5 ∓ 7.35	6.7 ∓ 2.68

No correlation exists normally between the carotenoid and vitamin A values of the liver and those of the blood. Blood tests were made on the day of slaughter, and the resulting carotenoid and vitamin A data compared with the results of the liver tests (table 1). The only time that low vitamin A stores were reflected by low vitamin A levels in the blood was during periods of rapid depletion of vitamin A stores of the liver without simultaneous intake of carotenoids or vitamin A, as was the case in the barn group without shark-liver oil supplement.

This limited observation was further substantiated by material obtained from the slaughterhouse. Livers and blood samples were collected from fifty-two animals, which were used in an experiment in which the effect of different vitamin A diets on the incidence of liver abcesses was studied.[3] The animals (Hereford cattle) had been kept in feed lots in three groups: high vitamin A diet, medium vitamin A diet, and low vitamin A diet. The means for the vitamin A stores found in each of the three groups and the means for the vitamin A levels of the blood of each group are presented in table 3, and the means plotted, in terms of percentage of decrease in vitamin A levels between groups,

[3] This study was conducted by Drs. H. S. Cameron and G. H. Hart. Acknowledgment is made for their kind cooperation in collecting these samples.

are shown in figure 2. It can be seen that a tendency towards a direct relationship between vitamin A stores and the vitamin A level of the blood exists only when the former falls below normal levels.

SUMMARY

Carotenoid and vitamin A values were obtained from liver samples of cows maintained in four dietary groups with different carotenoid and vitamin A intake. The vitamin A levels of the liver were significantly different among three of the four dietary groups, but similar for the shark-liver oil supplemented groups regardless of their basic diet. An optimum level for vitamin A storage was thus indicated.

Results obtained from livers of animals which had been maintained on a vitamin A enriched diet 6 to 8 months previously indicated a rather lasting effect on the vitamin A storage of a relatively short period of vitamin A feeding.

According to observations made on livers from vitamin A-starved animals and on samples obtained by partial hepatectomy, utilization of stored vitamin A first forces available carotenoid stores to be converted into vitamin A, thus decreasing the carotenoid level without decreasing the vitamin A level.

Similarly to the $\frac{A}{car}$ ratios observed in the blood, a typical relationship between carotenoid levels and corresponding vitamin A levels appears to exist in the liver. Changes in the ratio with changing carotenoid levels are probably caused by the tendency of the organism to maintain a constant vitamin A store. The $\frac{A}{car}$ ratio of the liver was found to be modified if the animal was fed vitamin A and in certain pathological conditions.

A tendency towards a direct relationship between vitamin A stores and the vitamin A level of the blood was found to exist only when the former fall below normal levels.

LITERATURE CITED

BARRON, N. S. 1942 Vitamin A and its relationship to some of the commoner diseases of farm animals. Vet. Rec., vol. 54, p. 29.

BRAUN, W. 1945 Studies on the carotenoid and vitamin A levels in cattle. I. Seasonal changes of the carotenoid and vitamin A levels and the normal carotenoid-vitamin A ratio of the blood. J. Nutrition, vol. 29, p. 61.

GUILBERT, H. R., AND G. H. HART 1934 Storage of vitamin A in cattle. J. Nutrition, vol. 8, p. 25.

RALLI, E. P., E. PAPPER, K. PALEY AND E. BAUMAN 1941 Vitamin A and carotene content of human liver in normals and diseased subjects. Arch. Int. Med., vol. 68, p. 102.

WITH, T. K. 1940 Absorption, metabolism and storage of vitamin A and carotene, with some remarks on the vitamin A requirements. Published by Einar Munksgaard and Oxford University Press, London, p. 1.

EDITORIAL REVIEW

THE REFINEMENT OF METABOLIC CALCULATIONS FOR NUTRITIONAL PURPOSES AND THE PROBLEM OF "AVAILABILITY"

ANCEL KEYS

Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis

(Received for publication August 21, 1944)

The supply and distribution of food under government control in war time makes great demands on nutritional science. The calculation of caloric requirements and the efficiency of food utilization assumes a new importance when the results form the basis of international food shipments and ration allotments. Even a very small percentage of millions of tons represents an impressive amount of food so there is an understandable desire to refine caloric calculations to the utmost. Such refinement involves several considerations.

Obviously the first step is to obtain more accurate analytical data on the composition of all foods which contribute significantly to the total diet. This task has been undertaken by groups of experts in different countries, notably England, Canada and the United States. Improved tables on food composition are now being made available in this country by the National Research Council. In general, such tables provide information on the food content in water, ash, fats and nitrogen as estimated by chemical procedures. The carbohydrate is obtained by difference, frequently with some correction for "fiber" or "digestibility."

Tables of food composition are used for the estimation of dietary adequacy and of human requirements in terms of specific foods. Our real concern is with the physiological results obtained from carbohydrates, fats and proteins and these may not be exactly indicated by the amounts of these substances measured by the usual analytical methods. McCance and Widdowson ('42) advocate corrections for "availability"of the carbohydrates and their ideas have influenced the views of the British experts so that American and British calculations for the caloric value of the current U.S.A. diet systematically differ by something like 5%.

Dr. L. A. Maynard ('44) has discussed the basis of classical caloric calculations and has shown that McCance and Widdowson overlook the fact that the standard calorie : gram factor for carbohydrates — 4.0 : 1 — already contains a reasonable over-all correction for "availability" of carbohydrate calories in the mixed diet. It will be agreed that in nutritional calculations we should like to use values which would more precisely allow for true availability, but the McCance and Widdowson figures appear to increase the errors for the total diet though they may improve the figures for some individual foodstuffs. However, even if the McCance and Widdowson tables were more properly constructed their use would not eliminate several sources of uncertainty.

The true problem of availability is not limited to carbohydrates nor to matters of simple digestibility. The physiological utilization of food is rarely important solely in terms of total calories or heat units. The uses of food for the purposes of growth, tissue maintenance and physical work are generally more important and these functions are not quantitatively related by an unvarying factor to the total heat. There are good reasons to believe that the efficiency of endogenous utilization of different nutrients is altered by the physiological destination of the energy involved.

There is no intention to question the equivalence of direct and of indirect calorimetry but the forms and places of energy conversions are of consequence. Neglect of these questions may lead to errors which, though relatively small, seem more significant as nutritional calculations become more exact. Unfortunately acceptable data in this field are few but several simple examples may be cited.

Both corn starch and glucose are completely "digestible" but their total heats of combustion per unit weight differ by 15%. For 100 gm. of these substances the total heats of combustion are 429.71 and 373.69 Cal., respectively. The first stage of the metabolism of starch is its hydrolysis, and part of the difference in total heat of combustion between starch and glucose is represented in heat of hydrolysis amounting to about 14.48 Cal. for 100 gm. of corn starch. If some of this hydrolysis takes place in cooking the food the corresponding heat of hydrolysis is completely lost. Ordinarily most of the hydrolysis takes place in the gastro-intestinal tract and this provides body heat which would appear in any total calorimetric measurement. But this energy is not "available" for any other purpose. It is clear that no single factor for the calculation of carbohydrate calories may be applied to both starch and glucose without significant error, and even if two different factors

are used it is necessary to specify the destination of the calories for rigid accuracy.

It is known that appreciable amounts of alcohol are readily metabolized to yield heat but it has been argued that calories of such dubious antecedents cannot be utilized to support growth or muscular activity. It now appears that alcohol at least may spare other nutrients so they may participate in growth (Mitchell, '35) and that alcohol may even provide energy for muscular contraction (Sommerkamp, '24; Grubbs and Hitchcock, '38). The efficiency in either process is unknown. The question is not entirely trivial when we note that alcohol may provide as much as 10 or even 20% of the total calories of the diet for some groups in northern and central Europe.

A more important example is the question of the efficiency of fats as fuel for muscular work. In this laboratory we have been able to confirm the findings of Krogh and Lindhard ('20) and of Bierring ('32) that, calorie for calorie, fat is less efficient than carbohydrate in the production of external work. The over-all difference is about 12%, that is about 112 cal. of mixed fats must be used to produce the same number of kilogram meters of external work as produced by 100 cal. of mixed carbohydrates. Since under the most favorable conditions muscular work is only about 25% efficient, it appears that the actual metabolic pathway — fat calories to muscular contraction calories — involves an excess wastage of about 16%. On the other hand, there is no difference if the calories are needed solely to maintain body temperature. The significance of these facts appears if we consider that on the average from 40 to 50% of all food calories are used in the production of muscular work and that in heavy industry and in military operations as much as 75% of the total calories may go into work. In contrast as little as 10% of the energy expenditure of the hospital patient may be devoted to muscular activity.

The few exact studies which have been made on the fuel of muscular work in man do not differentiate sufficiently between different types of fats and different types of carbohydrates. There are marked differences in rates of metabolism of different sugars and quite likely somewhat different metabolic pathways are involved. It is probable that these differences are reflected in differences in physiological economy. Nor do we have adequate information as to the relative efficiency of proteins in the support of muscular work. In terms of total efficiency what is to be done with the calories of specific dynamic action?

Under some ideal laboratory conditions the calculation of caloric requirements may be made with considerable accuracy but in real life

situations the errors are large. Even painstaking calculations with small homogeneous groups frequently lead to apparent absurdities. The recent study by Wiehl ('44) provides examples. Comparison of food intake versus elaborately computed "requirements" indicated that 55.3% of 272 private school pupils were undernourished but of these supposedly calorically deficient pupils only 11.1% were underweight while 47.7% of them were overweight by 7.5% or more. The incomplete data on public high school pupils suggest even greater discrepancies.

In this brief discussion it has not seemed necessary to consider the natural variability of foods which imposes an inherent limitation to the detailed accuracy of any tables of composition when applied to any particular sample or diet. The study of nutrition has made enormous strides in recent years but it is well to reflect on the limitations of present knowledge in making quantitative applications to man. There is some danger that revision of caloric tables to include "available carbohydrate" in the old sense may lead to a false sense of accuracy. Much more research, especially quantitative studies on man, is needed to create a true science of nutrition.

LITERATURE CITED

BIERRING, E. 1932 The respiratory quotient and the efficiency of moderate exercise measured in the initial state and in the steady state during postabsorptive conditions. Arbeitsphysiol., vol. 5, p. 17.

GRUBBS, R. C., AND F. A. HITCHCOCK 1938 The effects of small amounts of ethyl alcohol on the respiratory metabolism of human subjects during rest and work. J. Nutrition, vol. 15, p. 229.

KROGH, A., AND J. LINDHARD 1920 The relative value of fat and carbohydrate as sources of muscular energy. Biochem. J., vol. 14, p. 290.

MCCANCE, R. A., AND E. M. WIDDOWSON 1942 The chemical composition of foods. Third impression, revised. 150 pp. H. M. Stationery Office, London.

MAYNARD, L. A. 1944 The Atwater system of calculating the caloric value of diets. J. Nutrition, vol. 28, p. 443.

MITCHELL, H. H. 1935 The food value of ethyl alcohol. J. Nutrition, vol. 10, p. 311.

NATIONAL RESEARCH COUNCIL Tables of food composition giving approximate mineral and vitamin components of foods (on a 100-gm. basis). Committee on Food Composition of the Food and Nutrition Board. To be published.

SOMMERKAMP, H. 1924 Die Verwertung der Energie des Alkohols für die Muskelarbeit beim Hungernden. Pflüger's Arch., vol. 204, p. 528.

WIEHL, D. G. 1944 Medical evaluation of nutritonal status. XV. Caloric intake of high school students in New York City. Milbank Mem. Fund. Quart., vol. 22, p. 5.